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PARASITOLOGY

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VOLUME XXIV MARCH, 1932 No. 1

STUDIES ON EAST COAST FEVER. I. THE LIFE CYCLE OF THE PARASITE IN TICKS.

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(With Plates I–VII, containing Figs. 1–69 and Fig. I in the Text[1].)

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I. INTRODUCTION.

THE parasite of East Coast fever, *Theileria parva*, was observed by Koch in 1898, but it remained for Theiler (1904) first to characterise clearly the disease. Immunologically it differs from Redwater and anaplasmosis. Anaemia, jaundice and haemoglobinuria are absent, but there is a great enlargement of the lymph glands. As many as 90 per cent. of the erythrocytes may be infested

[1] Figures occurring on plates bear Arabic numbers, whilst text-figures bear Roman numbers.

with parasites. The mortality is usually fairly high (80 per cent.), but may, for some obscure reason, be quite low. Theiler (1911) also succeeded in transmitting the disease by the injection and insertion of emulsions and fragments of infected spleens and lymph glands—a line of work which was later extended by him in co-operation with du Toit (1928). The early literature on the disease has been critically reviewed by Walker (1909). A similar or identical disease has been reported in India, Macedonia and Transcaucasia, but the home of East Coast fever is the eastern side of the African continent from the Sudan to the Cape of Good Hope. It is a particularly heavy handicap to the settlers in Kenya Colony, where the problem of immunisation is being very actively investigated (Walker, 1926 and Walker and Whitworth, 1929). In spite of much careful work, no satisfactory method has been discovered for treating the disease, once it has been acquired, or of preventing it. Trypan blue, so helpful in Redwater, is useless. To keep the cattle free from the vector, *Rhipicephalus appendiculatus*, is extremely difficult in a thinly populated country in which the animals are not restricted to prepared pastures but are allowed to roam over large areas. More information concerning the pathogenesis of the condition and the life cycle of the causative organism, *Theileria parva*, is needed to serve as a logical basis for intelligently planned therapeutic and preventive measures. But up to the present time very little has been done in respect of the life cycle of the parasite, which most investigators have left severely alone.

Gonder (1910, 1911, 1911*a*), however, pursued investigations which led him to outline a definite sequence of stages in the development of *Theileria parva* in ticks. He reported that: "After the infected blood corpuscles have reached the stomach of the tick the parasites emigrate within the first half hour. A great number perish. Only the mature gametocytes grow into gametes and fuse with each other. The microgametes contain a distinct small nucleus similar to the centrosome or blepharoplast (Prowazek and Hartmann) of other organisms which acts as a stimulating agency for the development. The fertilised microgamete 'rounds off' after karyomyxis, from which the oökinete results, and, as in other blood parasites, takes the form of a retort, changing into that of a gregarine, and finally growing into an elongated oökinete. The oökinetes can be recognised by their activity; they double back and stretch out rapidly, and show contracting movements like gregarines." Gonder only found these oökinetes and the retort and rounded forms which immediately precede them in ticks examined "on the eve of moulting." He was able to find no transitions between them and certain clumps of minute forms which he regarded as infective sporozoites and the conclusion of the cycle in the tick.

The difficulty in estimating the value of Gonder's work is that substantiating details are conspicuous by their absence. He has never told us, for instance, the percentage incidence of the several stages, and he is vague regarding the position in the tick where they were found. Nuttall and Hindle (1913) failed in their attempt to confirm Gonder's work. They have made the statement that: "...although we have examined both smears and sections made from all

parts of the bodies of more than twenty infected ticks, we have not been able to detect any appearances which could not be found in normal ticks, when the tissues of both the infected and the clean ticks were searched with equal diligence. Considering that practically 100 per cent. of the ticks which imbibe the parasites are proved infective, the number we examined should have sufficed for their detection." Wenyon (1926) has declared that Gonder's "account was so obscured by such theoretical bias that it is difficult to separate fact from theory."

For many years we have been even more in the dark concerning the life cycle of the parasite of Redwater—another disease of cattle of economic importance. The life cycle of the causative organism, *Babesia bigemina*, was first studied by Koch in 1906. He reported the emigration of pear-shaped parasites from the red blood corpuscles into the stomach of the tick, which becoming amoeboid, extended long straight thorn-like pseudopodia. These amoeboid forms were said to associate in pairs and give rise to organisms possessed at first of two nuclei which later fused to form a single nucleus. Rather similar stages with spiky pseudopodia have also been recorded by Dschunkowsky and Luhs (1909, 1909*a*), but details are lacking and no information is available about their subsequent development. Very recently Dennis (1931) has published a preliminary account of certain studies on the life cycle of *Babesia bigemina* in ticks. He concludes that "the male gametes are not distinguishable from the female gametes," that "fertilisation apparently takes place by the isogametes becoming associated at the blunt anterior end and then fusing. Syngamy results in the formation of a mobile zygote or oökinete which soon leaves the lumen of the gut." He finds further that "the oökinetes penetrate the tissues of the thin gut wall where they may round up, grow and form sporonts varying from 5·0 to 12·0 microns in diameter, or may pass completely through the gut wall and enter the ovary where they invade the ova." The sporonts give rise in turn to sporokinetes which invade all the tissues of the tick and through sporogony form sporozoites.

Even the life cycle of the large piroplasm, *Babesia canis*, which causes malignant jaundice in dogs, is still largely a matter for speculation. Nothing has been done since Christophers' pioneer work with infective ticks in 1907. The parasites, like the other two mentioned, leave the red cells soon after their ingestion by the tick. In the gut of the tick Christophers found them to increase in size and become roughly spherical and then partly split in two, giving rise to a club-shaped body which he believed to pass through the wall of the intestine into the body cavity. In this new situation zygotes are said to develop, then sporoblasts and finally sporozoites. Wenyon (1926) thinks it "quite possible that some of the stages described as zygotes are actually tissue cells of the tick." An element of uncertainty will always remain until the problem is worked out with the aid of serial sections.

Large numbers of other piroplasms exist, but their life cycles are wholly unknown. When piroplasms are taken up by ticks feeding on sick animals, they

enter a kind of *terra incognita* only to reappear again in the blood of other animals and cause disease when the ticks feed on them in turn.

This investigation of the life cycle of the parasite of East Coast fever in ticks was undertaken in the hope that if the complete history of one piroplasm could be discovered the knowledge and experience thus gained would serve as clues to other life cycles. It was done at the invitation of Lord Passfield, Colonial Secretary at the time, acting for the Government of Kenya and on the recommendation of Sir Arnold Theiler. The experiments were made in the Government Laboratories at Kabete near Nairobi. To Major Eustace Montgomery, Veterinary Adviser to the Secretary of State; the Hon. Alexander Holm, Director of Agriculture; Major Brassey-Edwards, Chief Veterinary Officer; and Mr James Walker, Chief of the Veterinary Research Laboratory, we wish to express our thanks for many courtesies. We are also grateful to Mr R. Daubney, who was Acting Chief of the Laboratory during a part of our stay at Kabete, to Dr E. A. Lewis, and to Mr W. B. C. Danks, both of whom helped us in the actual conduct of the experiments.

As our work progressed it became clear that it would be necessary not only to trace the life cycle of the parasite in ticks but in addition to investigate the relationship between the injection of parasites, by the bites of ticks, into susceptible animals and the development in them of lesions characteristic of East Coast fever. Our presentation, therefore, is given in two parts: "I. The life cycle of the parasite in ticks," and "II. The behaviour of the parasite and the development of distinctive lesions in susceptible animals."

II. Material.

Six principal series of ticks (*R. appendiculatus*) were studied:

(1) *Infected larvae.* Collected 12. xii. 29 as nymphs from Bovine No. 4491 at the time reacting to E.C.F. (East Coast fever). Moulted to adults which were fed 22. ii. 30 on clean calf No. 5259, which died 20. iii. 30 of E.C.F. The larvae hatched from eggs of these adult females, presumably free from piroplasms since the evidence is convincing that the parasites of E.C.F. are not carried from generation to generation, were fed 22. vi. 30 on calf No. 5723 reacting to E.C.F. and 50 per cent. of whose erythrocytes contained parasites. They engorged and the observations detailed in Table I were made, indicating the fate of the ingested piroplasms. Tables I–IV are all constructed on the same plan. They give information as to the findings in serial sections. In column (1) the days after engorgement are noted. Sometimes the ticks were prepared immediately after they dropped off, so that no days (0) elapsed before examination. When the stages were particularly interesting several ticks were taken on the same day. In column (2) the number of individual ticks examined in serial sections is given and in the succeeding columns the presence or absence of parasites in special locations within the tick are indicated by plus (+) and minus (−) signs. Smears were also made daily, sometimes of many ticks. In

order to avoid complicating the tables, however, an asterisk is simply marked to indicate when and where parasites were observed.

(2) *Control larvae.* Collected 6. xii. 29 as adult females from Bovine No.4491 reacting to E.C.F. The larvae hatched from the eggs of these females were fed 16. vi. 30 on clean calf No. 5837. On their engorgement their daily examination

Table I. *Location of parasites in series of infective larvae.*

(1) Days after engorgement	(2) No. of ticks examined	(3) In red blood cells	(4) In lumen of gut	(5) In pedunculated cells	(6) In non-pedunculated cells	(7) As motile oökinetes	(8) In salivary glands
0	4	4+	3+	2+	–	–	–
1	1	1+	1+	1+	–	–	–
2	6	3+	5+	6+	–	–	–
4	2	–	2+	2+	–	–	–
5	.	.*	.*	.*	–	–	–
6	2	–*	2+*	2+*	–	–	–
7	5	–	2+*	3+*	1+	–	–
8	2	–	1+*	2+*	–	–	–
9	2	–	–	2+*	1+	–	–
10	3	–	1+	2+*	2+	–	–
11	2	–	–	1+*	1+	–	–
12	1	–	–	–*	–	–	–
13	6	–	–*	1+*	1+	–	–
14	2	–	–	1+*	–	–	–
15	2	–	–	–	2+	–	–
17	1	–	–	.*	–	–	–
20	1	–	–	–	–	–	–
21	3	–	–	–	2+	–	–
22	1	–	–	–	–	–	–
23	2	–	–	–	1+	–	–
24[1]	1	–	–	–	1+	–	–
26	2	–	–	–	–	–	–
27	1	–	–	–	1+	–	–
28	2	–	–	–	–	–	–
29	4	–	–	–	–	–	–
30	2	–	–	–	–	–	–
31	4	–	–	–	–	–	–
32[2]	1	–	–	–	–	–	–
33	1	–	–	–	–	–	–
34	3	–	–	–	–	–	–
35	8	–	–	–	–	–	1+
36	6	–	–	–	–	–	3+
37	4	–	–	–	–	–	3+
38	7	–	–	–	–	–	2+
39	4	–	–	–	–	–	1+
40	2	–	–	–	–	–	–
41	1	–	–	–	–	–	1+
42	1	–	–	–	–	–	1+
43	3	–	–	–	–	–	1+
44	2	–	–	–	–	–	–
45	2	–	–	–	–	–	1+
46	3	–	–	–	–	–	–

[1] Moulted to nymphae. [2] Fed on calf 5840.

in both smears and serial sections served as a control on the infected larvae; but neither this series nor the series of control nymphae is included in the tables in order to avoid unnecessarily complicating them.

(3) *First series infected nymphae.* Collected 10. xii. 29 as adult females from Bovine No. 4491 reacting to E.C.F. The larvae hatched from eggs were fed on clean calf No. 5438 and moulted to nymphs. These nymphs were fed

22. vi. 30 on calf No. 5723 reacting to E.C.F. On engorgement the observations detailed in Table II were commenced.

(4) *Second series infected nymphae.* Nymphs from the same source were fed 21. vi. 30 on calf No. 5740 reacting to E.C.F. and exhibiting parasites in about 70 per cent. of erythrocytes. Their study after engorgement with blood rich in piroplasms is indicated in Table III.

Table II. *Location of parasites in first series of infected nymphae.*

(1) Days after engorgement	(2) No. of ticks examined	(3) No. with parasites in red cells	(4) In lumen of gut	(5) In pedunculated cells	(6) In non-pedunculated cells	(7) As motile oökinetes	(8) In salivary glands
0	1	1+*	1+*	–	–	–	–
1	1	1+*	1+*	–*	–	–	–
2	3	3+*	3+*	2+*	–	–	–
3	1	1+*	1+*	1+*	–	–	–
4	1	1+*	1+*	1+*	–	–	–
5	.	.	.*	.*	–	–	–
6	1	–*	1+*	1+*	–	–	–
7	1	–	1+*	1+	–	–	–
8	1	–	1+*	1+*	–	–	–
9	1	–	1+*	1+*	1+	–	–
10	1	–	–*	1+	1+	–	–
12	1	–	1+*	1+*	1+	–	–
13	1	–	1+*	1+	1+	–	–
14	1	–	–	–	1+	–	–
15	1	–	–*	–	1+	–	–
16	1	–	–	–	1+	–	–
17	.	.	.	.	.*	.	.
18	1	–	–	–	1+	–	–
19	4	–	–	–	4+*	–	–
20	6	–	–*	–	5+*	–	–
21	1	–	–	–*	1+*	–	–
22	1	–	–	–	–*	–	–
23	1	–	–	–	1+	–	–
24[1]	3	–	–	–	2+	–	–
25	2	–	–	–	1+	1+	1+
26	1	–	–	–	1+	1+	–
27	1	–	–	–	–	1+*	1+
28	1	–	–	–	–	1+*	–
29	1	–	–	–	–	1+	–
30	1	–	–	–	–	1+*	1+
31	1	–	–	–	1+	1+*	–
32	1	–	–	–	–	1+*	1+
34	1	–	–	–	1+	–*	.
35	1	–	–	–	–	–	–
36[2]	.	.	.	.	.	.	.
37	4	–	–	–	–	–	–
38	2	–	–	–	–	–	1+
40	2	–	–	–	–	–	2+
41	2	–	–	–	–	–	1+
44	6	–	–	–	–	–	1+

[1] Moulted.

[2] Fed on calf 5199.

(5) *Third series infected nymphae.* Collected as adult females 14. i. 30 from sheep No. 3111. The larvae were fed on clean calf No. 5740 and moulted to nymphs. The nymphs were fed on calf No. 3586 reacting to E.C.F. (30 per cent. parasites), and studied in the same way after engorgement (Table IV).

(6) *Control nymphae.* Nymphs from the source just described were fed on

clean calf No. 5840 and their daily examination after engorgement afforded a control for the three series of infected nymphae.

In addition a number of ticks were studied at various times after the taking of blood containing parasites, but not in the same consecutive way or from pure cases of East Coast fever. Only 5, out of 167 of these examined in sections, contained parasites in their salivary glands.

Table III. *Location of parasites in second series of infective nymphae.*

(1) Days after engorgement	(2) No. of ticks examined	(3) No. with parasites in red cells	(4) In lumen of gut	(5) In pedunculated cells	(6) In non-pedunculated cells	(7) As motile oökinetes	(8) In salivary glands
6	2	–*	2+*	2+	1+	–	–
7	1	–	1+*	1+	1+	–	–
8	1	–	1+*	1+*	1+	–	–
9	.	.	.*	.*	.	.	.
10	.	.	.*	.*	.	.	.
11	1	–	1+*	1+*	1+	–	–
12	1	–	–*	1+*	1+	–	–
13	1	–	–*	1+*	1+*	–	–
14	1	–	1+	1+	1+	–	–
15	1	–	–*	1+*	1+*	–	–
16	.	.	.	.	.*	.	.
17	2	–	–	1+	2+*	–	–
18	10	–	–	2+	9+*	–	–
19	2	–	–	2+	2+	–	–
20	1	–	–	1+	1+*	–	–
21	1	–	–	1+	1+	–	–
22	1	–	–	–	1+	–	–
23	2	–	–	–	2+	2+	1+
24[1]	2	–	–	–	–	2+	–
25	1	–	–	–	–	1+	1+
26	1	–	–	–	1+*	1+	1+
27	1	–	–	–	–*	1+*	1+
28	1	–	–	–	1+*	1+*	1+
29	1	–	–	–	–	–*	1+
30	1	–	–	–	–*	–*	–
31	1	–	–	–	–*	–*	1+
32	1	–	–	–	–	–	–
33	1	–	–	–	–	–*	1+
34	1	–	–	–	–	–	1+
35[2]	6	–	–	–	–	1+	1+
36	5	–	–	–	–	–	2+
37	7	–	–	–	–	–	3+
38	5	–	–	–	–	–	3+
39	3	–	–	–	–	–	2+
40	11	–	–	–	–	–	4+
41	2	–	–	–	–	–	1+
42	4	–	–	–	–	–	2+
43	2	–	–	–	–	–	–
46	2	–	–	–	–	–	–

[1] Moulted.

[2] Fed on calf 5869.

III. TECHNIQUE.

The ticks were examined in three ways:

(1) Each day after engorgement, through moulting and feeding to engorgement on a susceptible animal, ticks were fixed in Bouin's fluid and serial sections of them were coloured by Giemsa's stain. The Bouin's fluid was of the following compositions: 75 c.c. saturated aqueous picric acid, 25 c.c. commercial formalin, and 4 c.c. glacial acetic acid.

After much experimentation it was found that complete serial sections could only be obtained in the case of ticks fixed after their chitinous coverings had been completely removed by careful dissection. The method used was to press the ticks gently into melted paraffin, in a Petri dish, which soon hardened and left their dorsal surfaces alone exposed. The ticks were then covered with a small amount of physiological salt solution and the dissection done with the aid of a binocular microscope. The chitin was removed from the dorsal surface by cutting all around the edge with a small sharp iridectomy knife and by severing with a specially curved knife the underlying attachment of the viscera

Table IV. *Location of parasites in third series of infective nymphae.*

(1) Days after engorgement	(2) No. of ticks examined	(3) No. with parasites in red cells	(4) In lumen of gut	(5) In pedunculated cells	(6) In non-pedunculated cells	(7) As motile oökinetes	(8) In salivary glands
1	2	–	2+*	2+*	–	–	–
2	1	–*	1+*	1+*	–	–	–
3	1	–	–*	1+*	–	–	–
4	1	–	–*	1+*	–	–	–
5	.	.	.*	.	.	.	
6	.	.	.*	.	.	.	.
7	1	–	–	–	1+	–	–
8	1	–	1+	1+	1+	–	–
9	1	–	–*	–	1+*	–	–
10	2	–	–	–	2+	–	–
11	2	–	–	–	2+*	–	–
12	1	–	–	–	1+	–	–
14	1	–	–	–	–	–	–
15	1	–	–	–	1+	1+	–
17	1	–	–	–	1+*	–	–
18[1]	1	–	–	–	1+*	1+	–
19	1	–	–	–	1+	1+*	1+
22	1	–	–	–	–	1+	1+
23	1	–	–	–	1+*	1+*	1+
24	1	–	–	–	–	1+*	1+
25	1	–	–	–	–*	1+	1+
27	1	–	–	–	–	1+	1+
28	1	–	–	–	–	–*	1+
29[2]	2	–	–	–	–	2+	1+
30	2	–	–	–	–	–	1+
31	1	–	–	–	–	–	–
47	1	–	–	–	–	–	1+

[1] Moulted.

[2] Fed on calf 5838.

to the chitin. The attempt was always made to remove the chitin from the soft parts of the tick rather than the soft parts from the chitin. With the same curved knife the ventral attachments of the viscera were likewise gently cut, allowing the entire contents of the tick to float out *en masse* in the salt solution. Even the chitinous envelope of larval ticks could be removed in this way without serious difficulty.

Engorged ticks, whether larvae, nymphs or adults, were the hardest to deal with, and in their case the technique was slightly modified for it was found that the making of a large slit in their chitin was immediately followed by the pressing out of their distended viscera through muscular contraction. They were therefore completely covered with paraffin which prevented contraction and expulsion of viscera when the first small incision was made after the removal

of a small part of the overlying paraffin. The remainder of the paraffin and the chitin beneath it was then carefully dissected away piecemeal and remarkably satisfactory results were obtained.

In order to follow the fate of the piroplasms in the serial sections, which were cut at 4 microns, it was necessary to intensify the blue coloration given by Giemsa's stain:

(*a*) The sections attached to the slides were passed through two changes of toluol and of absolute alcohol and one change of 95 per cent. alcohol to water.

(*b*) Instead of staining immediately, they were first treated for 30 sec. with 1 per cent. potassium permanganate. The excess of permanganate was then quickly removed by rinsing in tap water, and the remainder extracted by using a 5 per cent. solution of oxalic acid for about 30 sec. Finally the sections were gently washed in four changes of tap water, three changes of distilled water, and coloured overnight in diluted stain (3 c.c. of Giemsa's mixture to 100 c.c. of distilled water).

(*c*) The optimum degree of differentiation in alcohol was determined by experiment. It varies with the stage of the life cycle dealt with and the particular tissue under examination. Thus, with empty ticks ready to feed, a fairly long decolorisation in 95 per cent. alcohol is advisable, followed by passage through two changes of absolute alcohol. With recently engorged ticks, on the other hand, the 95 per cent. alcohol is to be omitted entirely, and the decolorisation done rapidly in the two changes of absolute alcohol. If attention is directed towards the parasite in the gut the differentiation should be slight, but if it is desired to bring out the stages of development in the salivary glands much stronger differentiation will be necessary.

(*d*) After differentiation the sections were passed through two changes of toluol and mounted in the kind of cedar oil ordinarily used with immersion objectives. By this technique the shades of blue are retained for a considerable length of time, partly owing to the mordanting action of the formalin in Bouin's fluid and partly due to the treatment with permanganate and oxalic described.

(2) *In smears.* The smears were allowed to dry in air. They were then fixed for about 5 min. in methyl alcohol, blotted with filter paper and covered with stain made up by adding one drop of Giemsa's stock solution to 1 c.c. of distilled water. The reaction of the distilled water was kept neutral by the addition of sodium carbonate, its neutrality being tested by bromo-cresol purple as suggested by Wenyon (1926). The stain was allowed to act for about 1 hour, when the slides were rinsed in tap water and quickly blotted with filter paper.

(3) *In dark field preparations.* These were made without the addition of physiological salt solution or of any fluid foreign to the tick. The contents of the tick, squeezed out on a slide, were covered immediately with a cover-glass. The preparation was then inverted so that the cover-glass was in contact with a flat layer of blotting paper, and with the slide above it, fairly strong pressure was brought to bear upon the preparation and in this way the tissue and body fluids of the tick were pressed into the thin layer necessary for dark field

examination. After pressing down in this way, the specimens were immediately ringed with vaseline to prevent evaporation. Displacement currents set up in the fluids soon subsided, and it was possible to make the observations in a background almost stationary. This technique was easily applied to all stages except unfed larvae and, to a lesser extent, unfed nymphae, with which it was necessary to act very quickly in covering the small amounts of material secured before they became dry.

Obviously, the speed with which the ticks developed depended on two principal factors; humidity and temperature. When not feeding they were confined in test-tubes plugged with cotton, which were kept in large uncovered battery jars in which the test-tubes rested on a thick layer of sand moistened with strong salt solution to prevent the growth of moulds. At first the test-tubes were maintained at room temperature which varied between 14 and 17° C.; but the development of the ticks was so slow that all the tubes were placed in a large incubator at a temperature of about 28° C. This change was made in the case of all our series of control and infected ticks, which at the time were of different ages. Those listed in Table I were transferred to this higher temperature on the 15th day after engorgement, in Table II on the 13th, in Table III on the 12th, and in Table IV on the 1st day after engorgement. The experiments were performed in the winter when the outside temperature was about 19° C. by day and 14° C. by night. The ticks were fed upon the ears of the animals enclosed in stout bags. These physical factors are given because if others repeat our experiments the rate of development of both ticks and parasites may be found to be very different from those which we describe unless the environmental conditions are roughly parallel.

IV. Observations.

(1) *Complicating factors.*

Before detailing our observations four complicating factors may be mentioned which added to our difficulties in the early stages of our investigations:

(*a*) The first was occasioned by the infestation of all ticks, larvae, nymphs and adults (both clean and containing *Theileria parva*) by a symbiont first reported by Cowdry (1925) in specimens of *Rhipicephalus appendiculatus* examined in South Africa. This symbiont is intracellular and is confined to the Malpighian tubules and ovaries, which, by contrast, never contain the parasite of East Coast fever. It is also hereditarily transmitted through the eggs. In sections it is not a source of error because it can easily be identified by its location; but in smears, where the cells are much broken up and topographical details are lost, its presence confuses the picture. It occurs in a variety of shapes and sizes, some of which are not unlike piroplasms. Like piroplasms it characteristically exhibits in its substance materials staining both red and blue by Giemsa's technique. When, as sometimes happens, the symbionts are in the form of spherules, the ground substance of which is coloured blue, and in which

there is seen at one side a sickle-shaped red-stained mass like a nucleus, the resemblance to a piroplasm is very close. Usually, however, the symbionts in smears can be distinguished from piroplasms by taking into consideration several criteria. The centrally placed red-staining material of the symbionts is generally made up of clusters of minute particles, whereas the nuclear material in most piroplasms is restricted to a single, sharply outlined, dense, homogeneous mass. The colour is also slightly different. In the symbionts it is lighter and tends towards crimson, while in the piroplasms it is darker and more purple. The cytoplasm of the piroplasms is often bluer than that of the symbionts and more clearly outlined by the cell membrane. In the smears the symbionts are more definitely intracellular than the piroplasms and are usually seen in much larger numbers, often made up of characteristic clumps. The properties of the background in which they are observed are also different. In the case of the symbionts typical Malpighian tubule cells may generally be distinguished and concretions characteristic of the tubules and rectal sac are readily identified. The piroplasms, on the contrary, are seen in a background which is more homogenous and of a slaty blue colour containing digestive spherules, clear vacuoles and perhaps a little pigment. In other words, the piroplasms are generally observed in material made up for the most part of broken down intestinal epithelial cells. The fact that Gonder does not mention the symbionts, though they must have been present with the piroplasms in his smears since they are present in 100 per cent. of the ticks, suggests the probability that he did not distinguish between these two kinds of organisms.

(*b*) The second complicating factor is less serious. It consists of the occurrence in all of our six principal series of infected and clean ticks of a protozoan parasite which does not appear to have been described in the literature. The parasite, at various periods in its development, inhabits the lumen of the digestive tract, the lining epithelial cells and certain wandering cells in the body cavity, which for convenience we call "macrophages." It is generally evenly pointed at both ends and from three to four times as long as it is broad. The largest forms are seen in the lumen of the gut, where they are naturally of a straw-yellow colour. Sometimes in epithelial cells coloured by Giemsa's stain they seem almost black, as illustrated in Fig. 5. But occasionally in epithelial cells much smaller apparently dividing forms are visible which possess distinct nuclei embedded in blue-staining cytoplasm. These often occur in the same cells with intra-epithelial growth stages of *Theileria parva*, but they can always be distinguished by their elongate shape and uniformly pointed ends, as well as by the fact that with Giemsa's stain their nuclei are coloured orange red in contrast with the darker more purplish red of the nuclei of the piroplasms. These parasites multiply actively in the macrophages, in which there are no piroplasms.

(*c*) Still another organism, having nothing to do with the parasite of East Coast fever, was observed in a few of our ticks in dark field preparations made of clean and infected larvae for as long as 10 days after engorgement. The

organism was about 3 microns long, 0·5 micron in diameter and very actively motile.

(*d*) But the greatest difficulty in our study was occasioned by the observation, contrary to the assumption of Nuttall and Hindle (1913), that 100 per cent. of the ticks fed on blood containing parasites do not become infected and thus capable of transmitting the disease. In our experience only a small and variable percentage of ticks retained the parasite. This made the detailed examination of a large number of ticks necessary.

(2) *The parasites in the blood on which the ticks fed.*

As a base line it is important to determine at just what stages these are in the life cycle. The range of variation in the parasites observed in the red blood cells of calf No. 9740 is illustrated in Figs. 29 and 30. In the former the red cells are included in order to give some idea of size and location. They are, however, omitted in Fig. 30 on account of spacial limitations, but the parasites represented were all intra-erythrocytic in position. The free forms so common in the gut of the tick were not observed.

The main point at issue is whether these parasites are agamonts (schizonts), gametocytes or gametes, or whether representatives of all three of these stages occur in the blood before it is ingested by the tick.

According to Gonder (1911): "The parasites produced by the gamonts represent the East Coast fever parasites which enter into the blood corpuscles. With the formation of these forms, the gametocytes, the development of the parasite in cattle reaches a definite point. It often happens that undeveloped gametocytes, which are still gamonts, enter the blood corpuscles and then continue their transformation. This is the reason why other authors refer to binary division and to reproduction in the blood of cattle. Sometimes cross forms are likewise observed in the blood which appear in the usual way in the organs when segmenting, *i.e.* when undeveloped gametocytes, viz. gamonts, enter the blood corpuscles. However, ordinarily a reproduction in the blood does not take place." In his later paper (1911*a*) Gonder specifies the difference between the micro- and macro-gametocytes as they exist in the red blood cells. The former are small comma-shaped organisms and the latter larger and plumper.

The morphology of the parasite in red cells has been subjected to a detailed investigation by Nuttall and Fantham (1910) without specific reference to male and female forms. It is more significant of the difficulty of making a statement as to sexual differences that three years later, well after the publication of Gonder's work, Nuttall (1913*a*) should summarise his studies again without any mention of males and females in the following terms: "While the parasite is very pleomorphic the commonest forms seen in stained preparations are ovoid, or rounded, and comma-shaped or clubbed. A proportion of the parasites are bacilliform. The appearance of the chromatin in some parasites suggests that multiplication may occur within the infected corpuscles, some of

which contain up to eight distinct parasites. If, however, multiplication occurs within the corpuscles it must take place very slowly or we should have observed it in the living parasite."

Until we have completed our studies on the parasite in the mammalian host which will appear as Part II of this contribution we do not wish to hazard definite statements as to the recognition of male and female forms in red blood cells.

(3) *Intra-erythrocytic parasites in the gut of the tick.*

Detailed dark field studies were made of the behaviour of these parasites. Careful observation of the precautions mentioned gave preparations in which the red blood cells were almost stationary and could be studied continuously for several hours. Since no foreign fluids were added and no temperature change was involved the osmotic conditions were approximately the same as in the lumen of the tick's intestine except for alterations, probably slight, caused by the unavoidable breaking up of many of the cells of the tick during the making of the preparation. The following findings are typical:

(i) Preparation made at 11.30 a.m. of a larva 24 hours after it dropped off, engorged, belonging to the series detailed in Table I. At 11.48 a red blood cell containing a single pear-shaped parasite was found. This cell was suitable for study because it was stationary and because its upper border was identifiable throughout the period of study by being slightly pointed. There were no Brownian movements. The following notes were made:

Hour.
12.10. Cell crenation began but was never marked.
12.47. Small areas of cell membrane became more highly refractile and their illumination seemed to flicker slightly.
1.15. Small highly refractile granule appeared and retained its position near pointed end of parasite.
2.24. Small granule became separated from parasite and disappeared.
2.30. Parasite was visible with difficulty and at
2.46. It disappeared.

Evidently the parasite did not move with anything approaching the agility described by Nuttall, Fantham and Porter (1910) in their examination of red blood cells taken from infected animals. Indeed it is questionable whether it moved at all of its own accord because the possibility of mass movements in the ground substance of the red blood cell could not be excluded. That such mass movements may have occurred is indicated by the fact that the small granule maintained the same position in respect to the piroplasm, and by the observation of slight crenation and of local changes in the refractive index of the cell membrane.

(ii) A day later, that is 48 hours after engorgement, an entirely different series of changes were observed. A preparation was made at 12.15 p.m. and at 12.20 a stationary red blood cell was selected containing what appeared to be five rather spherical piroplasms. The cell was definitely swollen through the

intake of water which caused marked Brownian movement exceeding that of the extracellular piroplasms in the vicinity. Changes in position of the parasites were therefore not of importance. Slight changes in the contour of the red blood cell were for a similar reason discounted. Chief attention was paid to alterations in the shape of the parasites, four of which were seen to elongate to form bacillary structures, but division was not observed.

It is not possible to gauge the time of liberation of parasites into the lumen of the gut from the red blood cells by the observation of free forms, because extracellular parasites are to be observed from the time blood is first ingested and persist in the gut longer than the red cells. The only feasible way is to determine how long parasites can be recognised within the erythrocytes after the ticks drop off, engorged with blood. When no more can be seen obviously all of them have been discharged. Reliance was placed entirely on smears of ticks stained by Giemsa's method because our sections were made from ticks fixed in Bouin's fluid which, owing to its content of acetic acid, usually destroys red blood cells while preserving the parasites very satisfactorily. But there are cases in which the reds are well fixed by this method.

Table V. *Duration of survival of parasitised erythrocytes in gut of ticks.*

Days after removal	No. of ticks examined in smear preparations	No. showing parasites in intact red cells	Ticks containing parasites in red cells %
0	6	6	100
1	8	6	75
2	6	6	100
3	4	1	25
4	8	2	25
5	7	0	0
6	7	1	14
7 and up	—	000	000

Data are presented in Table V which indicate the presence of intra-erythrocytic parasites only up to the sixth day. This observation is rather at variance with the statement of Gonder (1911): "That the parasites quit the blood corpuscles as soon as they enter the stomach of the tick, generally within a couple of hours." Persistence as long as this is, however, the exception rather than the rule. Great variation was noted. Thus, on the second day all of the parasites were extracellular in two of the eight ticks examined. As yet unknown factors control the rate of liberation of the parasites from the red blood cells. There are, however, two principal possibilities: (1) that the ticks themselves differ, though they may be brother or sisters reared under almost identical conditions—it is significant in this connection that Nuttall (1913) has emphasised the variations in size and structure of *R. appendiculatus*, the very species which we employed; (2) that they have taken blood differing in quality, perhaps more venous, or more arterial. It is our impression that parasites are liberated somewhat more rapidly in engorged larvae than in engorged nymphae.

The mechanism of liberation is not clear. In our dark field examinations

which were made daily over this period a parasite leaving an intact erythrocyte was only observed on one occasion. The cell in question contained a single organism which changed its position in a sluggish way. After being held under continuous observation for 15 min. it approached the cell membrane and came in contact with it. When this happened the local appearance of the membrane changed. Instead of appearing as a thin luminous line the thickness of the illuminated area at the point of contact increased three or fourfold. The parasite when it touched this brightly shining segment of the cell membrane suddenly disappeared and could not be followed in the surrounding fluid. Very quickly the cell membrane regained its original appearance. In stained smears no examples were seen of parasites in the act of emerging from red blood cells despite careful search. It is likely that the chief factor in the liberation of parasites is the destruction of the cells containing them.

Since it often takes several days for the ticks to engorge, it is obvious that some of the red blood cells in their gut must have been present longer than others, which makes it impossible to state precisely how long individual cells survive after ingestion. During the first few days the parasites are being freed fairly continuously, so that many stages in their subsequent history will be present in the same specimen.

(4) *Parasites free in the lumen of the gut.*

As indicated in column 4 of Tables I–IV, free parasites were found in the lumen of the intestine up to 10, 13, 14 and 8 days respectively in our four series of infected ticks. This observation was made through the use of serial sections because in the preparation of smears the epithelial cells lining the gut are ruptured and one cannot tell with certainty whether the parasites observed in smears were previously free in the lumen or were within epithelial cells. In the earlier specimens of the several series some of these parasites probably occupied a position within red blood cells, and consequently, though within the lumen of the intestine, were not free in the sense of being extracellular. This reservation is made for the reason already stated that the acetic acid in the fixative (Bouin's) generally destroys the erythrocytes. It is safe to say, however, that parasites seen in sectioned material within the lumen after 7 days, when the examination of smears (Table V) failed consistently to reveal intracorpuscular parasites, are in fact free in the lumen.

The first two lines (Figs. 29 and 30) represent parasites in the blood on which the ticks fed, as has been described already. The third (Fig. 31), fourth (Fig. 32), fifth (Fig. 33), sixth (Fig. 34) and seventh (Fig. 35) illustrate forms observed at the time of engorgement and 3, 5, 8 and 12 days thereafter. Since all these figures were drawn from smears, information is not available as to the location of the several forms of the parasite in the tick, whether free in the lumen or within pedunculated or non-pedunculated epithelial cells. We shall return to their consideration later. At present we are concerned with parasites

definitely within the lumen as seen in sections. These are shown 2 days after engorgement in Fig. 36, *a–w*, and 5 days later in Fig. 38, *o–ac*.

Some parasites within the lumen degenerate *in situ*. As evidence for which statement may be cited the progressive loss in the affinity of their nuclei for stains to the point where the nuclei can no longer be distinguished and the parasites persist simply in the form of tiny basophilic masses (Fig. 36, *e* and *f*, and Fig. 38, *w*, *y* and *z*). Before these masses finally disappear their shape usually becomes irregular.

While many of the degenerating and healthy parasites in the lumen are distributed sparsely and fairly evenly in the contents of the gut the majority exhibit a marked tendency to gather together in clumps. These clumps are occasionally 20–30 microns in diameter and are made up of hundreds of individual parasites. Quite frequently two clumps in the same section differ in the size of the parasites of which they are composed.

In cases where the parasites are large they are of roughly spherical shape (Fig. 36, *b* and *n*, and Fig. 38, *u* and *ab*). Their nuclei are coloured deep crimson by the stain and their cytoplasm a blue like that of a robin's egg. The nuclei are placed to one side and are variable in shape. Single spherical nuclei are comparatively rare. Crescent-shaped forms predominate and are usually so closely applied to the cell membrane that cytoplasm can only be seen on the side of their concavity. In about 5 per cent. of the parasites the nuclei are constricted equatorially and partly divided into two parts (Fig. 38, *s* and *t*). Very rarely parasites are found containing two nuclei (Fig. 38, *ac*), though, as we shall report later, such formations are of more common occurrence in the case of parasites within the epithelial cells. The volume of the cytoplasm of these large parasites exceeds that of the nuclei. In parasites with crescentic nuclei the cytoplasm in the nuclear concavity is often distinctly less intensely stained presenting an appearance faintly reminiscent of the cytoplasm of tiny plasma cells. Cytoplasmic constrictions corresponding to the nuclear constrictions were not noted. It is possible that these relatively large forms are female gametocytes undergoing division to form gametes and that the gametes are indistinguishable in sections from the gametocytes; but we have no supporting evidence.

The small parasites making up other clumps are equally distinctive. Each possesses a single spherical nucleus which is stained about the same colour as in the large forms but more intensely (Fig. 36, *i*). A relatively small amount of cytoplasm is present, the total volume of which is usually less than that of the nucleus contained in it. The cytoplasm may surround the nucleus fairly evenly or may be drawn out into a tail-like process. Such parasites may be males; but again we have no evidence. It is to be noted, however, that morphologically and tinctorially they are, as far as we can see, undistinguishable from the small parasites seen in the red blood cells of the calf before their ingestion by the tick (Fig. 29, *b*). They are also regularly met with in smears of ticks made up to 12 days after engorgement.

In addition to this accumulation in clumps, both large and small parasites gather together on the surface of the epithelial cells which line the lumen of the gut.

(5) *Parasites in pedunculated epithelial cells of the gut.*

It is known from the investigations of Nordenskiöld (1908) and Robinson and Davidson (1913) that after engorgement with blood certain epithelial cells of the stomach and alimentary caeca project further into the lumen than their neighbours. The latter authors worked with *Argas persicus* and found that: "After engorgement, the epithelium becomes thicker, and here and there individual cells become elongated so as to protrude into the lumen of the organ beyond the general surface of the stomach wall....These pseudopod-like protrusions of the epithelium assume a more or less spherical form, but always remain attached to the basement membrane by their now pedunculate bases. The nuclei of these epithelial cells, which in unfed individuals are situated near the basement membrane, move toward the free margins of the cells and take up their position in the protruded spherical extremities. The cell contents undergo considerable changes. Before engorgement the cytoplasm is finely granular and, as mentioned above, the nuclei are situated in the bases of the cells. After engorgement, and while active digestion is in process, the proximal portions of the cells develop a very striking reticular structure, and the distal portion of each cell, more particularly those which have elongated to form the pseudopodial protrusions, become densely packed with large spherical granules which are deeply stained by acid dyes." They believe that these spherical granules "are the actual nutrient products elaborated from the contents of the stomach, and passed on in this form to the coelomic spaces surrounding the gut, to be carried to the different parts of the body by the blood stream." Their illustration, Pl. XVII, fig. 2, shows only slight protrusion and not so much difference in structure between protruded and non-protruded cells, as is represented in the figures of Nordenskiöld. Though these two cells are of the same fundamental type and probably differ only because they happen to be, when observed, in a different phase of their functional activity, the way they behave in respect to ingested parasites is so divergent, that we propose to describe them under separate headings.

The pedunculated epithelial cells have other distinguishing criteria in *R. appendiculatus*. The spherical granules are larger and more conspicuous than in adjacent cells. In our preparations they are coloured faintly blue or light pink, depending upon the mordanting action of the fixative and the differentiation of the stain. The nuclei are often shrunken and show signs of degeneration. In contrast with the other cells they contain during the first few days not only the parasites but also polymorphonuclear leucocytes, eosinophilic leucocytes, lymphocytes (sometimes with Koch bodies), a few Koch bodies and platelets.

Parasites are found in the distal portions of these pedunculated cells from the first to the fifteenth day after the ticks drop off, engorged. They are most

numerous between the third and the ninth days. Parasites in this situation as seen in sections are illustrated 3 days after engorgement in Fig. 37, *a–f*, and 7 days after engorgement in Fig. 38, *a–n*. Whether they gain entry by virtue of their own motility and powers of penetration or are phagocytised by the epithelial cells, or whether both factors are operative, we have not been able to determine. They occur both in the substance of the spherical granules and in the very lightly stained, slightly granular matrix in which the granules are embedded. Many of them are undergoing degeneration (Fig. 38, *h*), but a considerable number take the stain with avidity. In shape the latter are extremely varied. The large and small forms mentioned as occurring free in the gut are easily recognisable. The polymorphism is, however, much more marked than in the gut. Appearances suggestive of division of both large (female?) and small (male?) forms are frequently encountered. Nuclear division in the former results in two masses of chromatin generally alike in size and functional properties, but occasionally, as may be more clearly distinguished in the non-pedunculated cells (see Fig. 38, *ar–av*), one mass may be larger than the other (Fig. 38, *c* and *ac*), which may indicate nuclear reduction, *i.e.* formation of female gametes. In the case of the smaller forms no signs of unequal division were detected.

Very careful search was made for fertilisation stages without conspicuous success. Large and small parasites were, however, occasionally observed in contact (Fig. 37, *d*, *e* and *f*), but it was difficult to tell whether this was merely optical superposition or whether actual union was taking place. Such appearances were detected in 0·1 per cent. or less of the parasites. Cases like those illustrated required hours to find.

In these cells containing highly developed spherical granules other and larger parasites were seen, which we believe to be the zygotes resulting from fertilisation (see Fig. 38, *i* and *j*). They are of rare occurrence, usually near the nuclei of the epithelial cells, and it is extremely doubtful whether any of them survive, as they do with considerable regularity in the other epithelial cells about to be described.

(6) *Development of parasites within non-pedunculated epithelial cells of the gut.*

These epithelial cells constitute the vast majority and do not require characterisation, for they differ from the others in ways which have already been specified. Since they do not as a rule exhibit in addition to the parasites a variety of formed elements, like leucocytes, obviously taken in from without, it seems likely that in their case at least the parasites enter through the exercise of their own powers of penetration. Having entered, the parasites come to rest in the cytoplasm just within the cell membrane. These parasites are seldom as numerous as those in the pedunculated cells, and their morphology is less varied. Fig. 38, *ad* to *av*, shows their appearance in a nymph 7 days after engorgement. Small, possibly male parasites, like those which are abundant both in the lumen and in the pedunculated cells, are conspicuous by their absence,

though some are occasionally seen. This fact taken alone is weak evidence that among the parasites will be some which are zygotes, the males having disappeared through union with the females. The close apposition of large and small parasites was occasionally seen, as has been illustrated as occurring in the pedunculated cells. Some large parasites divide (Fig. 38, *ar* to *av*), and these may represent the last prefertilisation divisions of the females. The new forms which develop in these epithelial cells to the exclusion of all other are, we believe, in fact, zygotes. At first they are large parasites provided with a single distinct nucleus and much basophilic cytoplasm (Fig. 39).

Before detailing their subsequent history, for there can be no question that they alone carry on the life cycle, we wish to return to our smears, in which all topographical considerations are lost, and present some supplementary evidence relative to the occurrence of reduction divisions in the change from gametocytes to gametes and to the process of fertilisation. We have deferred doing so until now because we thought it was first important to give some idea as to what was going on simultaneously in the three locations described, viz. the lumen, pedunculated and non-pedunculated epithelial cells, which could only be reached by a close study of sections. In the smears the important element of location is obviously lost. The smears have, nevertheless, certain advantages as compared with sections. Entire parasites are always seen—not, as in sections, whole parasites mixed with many incomplete slices of other parasites cut at various angles. The parasites appear also to be larger, because in making the smears they were somewhat flattened as compared with the general shrinkage resulting from the action of Bouin's mixture on all sides of parasites suspended in a fluid medium.

Stages suggestive of equal division of small and of large forms were observed abundantly. As in sections, unequal (reduction) division of the small parasites to form gametes capable of fertilisation was not seen, but with parasites so tiny the degree of inequality may be so small as regularly to escape detection. Some signs of unequal division of the large ones were observed. They consist only of fairly robust parasites containing two rather large masses of chromatin which are at opposite sides of the parasite and seem to be separating. Indications of the union of small and large forms like those already reported in sections were discovered after prolonged search (Fig. 33, *e*, *m*, *n*, *o* and *p*). The resulting zygotes are much larger (Fig. 33, *o*, Fig. 35, *k–p*). Unlike the other forms of the parasite they cannot be detected immediately after engorgement. Considering the large number of fertilisations which must take place to provide the zygotes to carry on the cycle it is surprising that the process is so difficult to prove. Many attempts were made without success to observe the act of fertilisation by living parasites in dark field preparations. Let us now return to the sections.

In the absence of convincing proof we realise that we are not justified in using the word "zygote." We do so only because the available evidence seems favourable to this interpretation. The two dissimilar forms (male and female gametes?) are replaced in the specimens by this single new form which carries on the cycle. We have to do with a single morphological type, in place of two, which is present not in three locations (lumen, pedunculated and non-pedunculated epithelial cells) but only in one, namely, the non-pedunculated cells of the gut. From this point on we can speak of what happens with greater assurance. The zygotes are never as abundant as the stages of the parasite which preceded them. When present they can usually be quickly detected by examination with a low-power objective, but long search of serial sections may be required to

reveal them. The cells are unevenly infested, some are devoid of zygotes, others contain quite a number, but it is unusual to observe more than 20 or 30 in a section through a cell 4 microns in thickness, whereas several hundreds of parasites are often seen undergoing destruction in a section of a single pedunculated epithelial cell. The small area of cytoplasm occupied by these zygotes differs from the remainder by staining less intensely and by being reticulate or vacuolate (Figs. 1–4). It appears to be more watery.

The parasites soon lose their spherical contours and become angular, almost spidery, in shape. The appearance as seen in a nymph 18 days after engorgement is represented in Fig. 40, *a–j*. The sides of the zygotes are indented by clear droplets as indicated in Figs. 40, *g* and 41, *a*. Such droplets are much more abundant in immediate contact with the zygotes than in parts of the same cells which do not contain zygotes. It is through the unequal pressure thus exerted that the yielding surface of the zygotes is pushed in, in some places, and pushed out between the droplets, in others. Distinctly light blue and red staining materials can no longer be made out and the organisms become coloured a uniform dull blue. They look as if they were being digested by the epithelial cells containing them. At first we feared that at this point we were losing the parasites. Though actual digestion is probably in fact the fate of many, some survive and increase in size despite this digestive action of the cells containing them.

In Fig. 41 other further advanced zygotes are shown from a different tick, but the same number of days after ingestion of infected blood. Some of these zygotes are noticeably larger (*a–d*).

Their growth can best be estimated by comparing the zygotes in Figs. 1 to 5 which are photomicrographs taken at a magnification of 1400 diameters. The diameter of the zygotes increases 300–500 per cent. While examining Fig. 5 it is important not to confuse the regular ovoid, intensely black masses to the left and just within the cell membrane, which are other Protozoa already mentioned among the complicating factors, with zygotes which are more irregular in shape, less strongly stained and more deeply situated in the cell. A higher magnification (× 3250) was used in making the drawings for Figs. 29–69. In a section of a single cell the zygotes seldom seem to be of the same size, but some are probably only partly included. It is unlikely that there is really any great difference *inter se*. During growth in size there is no definite evidence of an increase in number, though some zygotes are equatorially constricted (Fig. 40, *h* and *j*). The processes of neighbouring zygotes are often in contact, but it could not be ascertained whether there is at any time actual protoplasmic continuity between them, as suggested by their appearance in Fig. 40, *i*. With the increase in volume two other changes occur: inhibition of digestion and reappearance of nuclei.

The cessation of digestive processes, as far as the parasites are concerned, is marked by a return to smooth outlines and a reduction in the number of droplets indenting the zygotes. Thus the contours of the zygotes depicted in

Fig. 41, *a* to *d*, are much more even. One of them, *c*, illustrates how the shape of the zygote is governed at this stage by contact with the surfaces of large digestive spherules which are convex in relation to it.

The nuclei reappear as small areas of the zygotes which stain at first faintly red in a blue background. This rapidly changes to the same bright crimson hue of the nuclei of the free parasites in the gut from which they are descended. It is best illustrated in the coloured drawings, Fig. 41, *e* to *h*, but can be detected by a close study of the zygote below and to the left in the photomicrograph, Fig. 6. It will be observed that as they develop the nuclei tend to be surrounded by a kind of halo of cytoplasm which is less deeply coloured than the rest. The nuclei may be single (Fig. 41, *e*) or multiple (Fig. 41, *h*). In the latter case they are smaller. We have not made accurate counts, but we estimate that fully 80 per cent. of the zygotes (which are changing into the next stage called "oökinetes") possess single nuclei, about 15 per cent. two nuclei and 5 per cent. three or four. Zygotes with five nuclei were not seen, though they may occur.

We have relied almost entirely upon sections in the study of these growth stages of the zygotes. Without sections and employing only smears we would probably have lost the parasite during this phase of its development, for it is extremely difficult to identify in smears. It looks, in smears, something like a degenerating nucleus of some cell belonging to the tick except that its colour, when stained by Giemsa's method, is pale blue without a shade of red; whereas the nuclei of the cells of the tick are deep purple. Reference to column 6 of Tables I–IV will show the days after engorgement when such forms and their predecessors in non-pedunculated cells were observed in sections and in smears, the former being indicated by + signs and the latter by asterisks. The absence of a positive finding in smears does not signify the absence of zygotes, but rather that the preparations have not been examined long enough. Though smears were made daily of ticks of all four of the infective series and of the controls we concentrated our attention upon the sections, since they were in every way more helpful.

(7) *Formation of motile "oökinetes" and their migration to the salivary glands.*

In an arbitrary way this development may be dated from the time when the length of the zygotes becomes more than twice their diameter. It occurs from the day before moulting, through the actual moulting and as late as the thirty-first day after engorgement.

Oökinetes forming within zygotes are illustrated in Figs. 42, 43 and 44. It will be observed that the substance of the zygote is drawn together in a central mass. This central mass constitutes the future oökinete. It is usually definitely elongated and contains a distinct nucleus near one end. The space between the developing oökinete and the original wall of the zygote becomes vacuolated and stains progressively less intensely. It seems to contain a watery fluid. The limiting membrane of the zygote becomes stretched out as the oökinete

increases in length (compare Figs. 42 and 43), or else the growing oökinete becomes folded as indicated in Figs. 45 and 11. Each oökinete appears to develop in a kind of capsule formed by transformation of the zygote. Finally the wall breaks (Fig. 44) and the oökinete is liberated. These changes were first observed in sections.

Smears were useful for purposes of confirmation. Differentiation of nuclei and elongation during oökinete formation, as observed in smears, are illustrated in Figs. 8–10. Because topographical details are lost in making smears certain criteria useful in identification may be mentioned. While still roughly spherical (centre Fig. 8) the parasites look something like faintly stained nuclei belonging to the cells of the tick; but in air-dried smears, fixed with methyl alcohol and stained by Giemsa's method their coloration and structure are typical. They are stained a faint rose pink in contrast with the deep crimson of the abundant chromatin in the nuclei, which by contrast is often very densely distributed along the nuclear membrane. They contain a little irregularly placed finely particulate matter which is very different from the coarse masses of chromatin characteristic of the nuclei of the tick. They generally occur in a background made up largely of epithelial cells broken up in the making of the smear. Finally, the parasites in this particular stage are almost invariably identifiable in smears by the peculiarities of their own nuclei. A parasite is seen in about the centre of each of the three figures mentioned. In Fig. 8 it is rounded, and along its lower margin the single nucleus present is almost heart-shaped; in Fig. 9 the parasite is longer and the shape of the nucleus still more typical; while in Fig. 10 the lengthening is more marked, the nucleus tending to be ovoid. In all three the size and staining qualities of the nucleus are fairly constant.

Fully formed oökinetes are shown in sections by a drawing (Fig. 49) and by a photomicrograph (Fig. 7), and in smears by several photomicrographs (Figs. 11–14). The oökinete represented in Fig. 49 is not actually twice the size of the others. It is merely that the drawings were made at a magnification of 3250 diameters—more than twice that used for the photomicrographs. Although oökinetes were studied in the living state actual measurements were, unfortunately, not made. Assuming, however, that the shrinkage in smears, in which they are flattened out on the slides in the process of drying, is slight it would appear that the length of fully developed oökinetes is about 15 microns and their maximum width approximately 3·5 microns.

The oökinetes are about four or five times as long as they are broad. The anterior end is bluntly rounded and sometimes bears a small projection staining rather differently from the rest (Figs. 44 and 49). The nucleus is placed well anteriorly and is usually solitary, though as many as two may occur in a single parasite. Very often the nucleus is heart-shaped and situated in an area of cytoplasm which takes the stain less intensely than the remainder. Occasionally it is rod-like, almost filamentous (Fig. 12). Posterior to the nucleus the cytoplasm sometimes stains particularly strongly (as indicated in Figs. 42, 43

and 45). The posterior part of the parasite tapers to a fine point and never contains nuclear material.

In spite of daily dark field examinations only once was a moving oökinete seen in a preparation of a female tick belonging to the series listed in Table IV and taken the twenty-third day after engorgement. The parasite in question exhibited the typical morphology already mentioned and glided in an effortless sort of way through the preparation, blunt end first, for a distance about equal to its length and then came to rest; it then remained stationary for half an hour when the observation ended. In the same specimen a bent oökinete (like Fig. 11) was observed for 75 min.; it made no appreciable movement. Preparations of two male ticks of the series detailed in Table IV were examined on the twenty-eighth day, one by dark field and the other by direct illumination after the addition of a very small amount of methylene blue. Both exhibited oökinetes, but in neither of them did the oökinetes show motility. In the second specimen the bodies of the oökinetes were tinged faintly blue and their nuclei were coloured a little more deeply.

The actual act of penetration of the oökinetes through the wall of the intestine into the body cavity was not seen, but on many occasions they were observed in sections free in the body cavity near the salivary glands. The delay between their formation and entry into the salivary glands is short. In the series listed in Tables II and III they were first detected within the salivary glands the same day that they were discovered elsewhere, while in the third series of infected nymphae listed in Table IV they were not identified in the salivary glands until an interval of 7 days had elapsed. Some of the oökinetes, however, persist in the gut and body cavity long after their fellows have entered the salivary glands. This is indicated by the observation of oökinetes 9 days (Table II), 12 days (Table III), and 7 days (Table IV) after the first intrasalivary parasites were seen. The piercing of the cell walls of the salivary gland cells by the entering oökinetes was not observed.

We have employed the designation "oökinete" in our description of these large migratory forms of the parasite because it is a word in general use and we do not wish to invent new terms. It does not follow, however, that our oökinetes correspond exactly with others described in the literature. Their resemblance to the so-called "club-shaped bodies" first observed by Christophers (1907) in his researches on the life cycle of *Piroplasma canis* in the tick is very striking. They are quite different from the "oökinetes" of *Theileria parva* described by Gonder (1911*a*) and illustrated in his Figs. 48 and 49. The degree of similarity between our oökinetes and the "motile zygote or oökinete" mentioned by Dennis (1931) in his preliminary account of the cycle of *B. bigemina* in ticks will be difficult to establish until he publishes a detailed account of his findings.

(8) *Development of parasites within the salivary glands.*

In this location parasites were observed over a long period of time, as indicated in Tables I–IV, namely:

(i) From 35 days after engorgement, 11 days after moulting and 3 days after feeding on a susceptible animal to 45 days after engorgement—an interval of 10 days.

(ii) From 25 days after engorgement, 1 day after moulting and 11 days before feeding on a susceptible animal to 44 days after engorgement—an interval of 19 days.

(iii) From 23 days after engorgement, 1 day before moulting and 12 days before feeding on a susceptible animal to 42 days after engorgement—an interval of 19 days.

(iv) From 22 days after engorgement, 4 days after moulting and 7 days before feeding on a susceptible animal to 47 days after engorgement—an interval of 25 days.

Data obtained from the first series infected as larvae are not so complete during the first 2 or 3 weeks of observation, as in the case of the infected nymphae, because at that time we had not become proficient in dissecting off their chitinous covering.

During the lapse of about 3 weeks many changes occur in the parasites in the salivary glands which for convenience we shall describe in periods: (*a*) before placing the ticks on susceptible animals; (*b*) during the first 3 days on susceptible animals; (*c*) from 4 to 18 days on susceptible animals.

(*a*) *Before placing the ticks on susceptible animals.*

In our four series of nymphae which engorged with blood containing parasites oökinetes were first observed in salivary gland cells: 1 day after moulting to adults, 1 day before moulting and 4 days after moulting. In the vast majority of cases a single cell is parasitised by a single oökinete; but, as will appear later, there is evidence that in rare instances at least two oökinetes, perhaps more, may enter one cell and develop in it. At the time of penetration the cells themselves are still small; not very much larger than the oökinetes. An oökinete is illustrated in Fig. 50 within a salivary gland cell, whose nucleus is not included in the section. This parasite is still somewhat elongated and is surrounded by a clear halo of cytoplasm which very characteristically does not take the stain. It will be noted that the nucleus is tinged the same crimson colour as the nucleus of a mature oökinete (Fig. 49) and that it is likewise possessed of a halo in both instances. The cytoplasm is coloured the same quality of blue, but much more deeply. As development proceeds this increase in affinity for stains becomes still more marked (Figs. 51–55). In order to bring out the structure of these intracellular oökinetes it is necessary to modify the technique by destaining the preparations to the point where the free oökinetes are almost colourless. Unless this precaution is taken many of the early intraglandular forms will remain undiscovered. While this change in staining reaction undoubtedly is the expression of a fundamental alteration in the parasite it is possible that it is enhanced by the new surroundings in which the parasite now finds itself. Instead of being a long, thin motile form free in the body cavity and unrelated to materials possessed of a strong affinity for stain, it is shorter

and thicker and it is located in the salivary gland acini which stain intensely. In other words, both by change in shape and position it is likely now to hold the stain better than before.

Fig. 51 illustrates two salivary gland cells of a nymph 23 days after engorgement. The nucleus of the uppermost cell is somewhat shrunken and its staining reaction is altered. This may be due to the influence on the nucleus of the adjacent parasite which is readily identified by the crimson staining of its nucleus, the blue tint of its cytoplasm and the halo of clear cytoplasm which surrounds it.

A similar stage is shown in a photomicrograph (Fig. 15). Slightly to the left of the centre is situated a salivary gland acinus, in the right lower sector of which an intracellular parasite can be detected. It is rather larger than the nuclei of the salivary gland cells. Its own nucleus is already visible as a small spherical mass which protrudes from its upper right margin. Having in mind the difference in magnification of photomicrographs and drawings it is evident that the parasite itself was of about the same size as the one shown in Fig. 51.

As development goes on the parasite—for it is a question how long we can go on calling it an oökinete—becomes more rounded, loses its nucleus and stains more intensely blue (Fig. 52). A rather later stage is shown in Fig. 54, but in this case the salivary gland cell is omitted in order to save space. There is also considerable growth which may be estimated by comparing Figs. 50–55 and 15–17. The increase in diameter amounts to at least 100 per cent. A stage like Fig. 54 is shown in the centre of the photomicrograph, Fig. 16, in which the repeatedly mentioned halo is quite apparent. The parasites retain this general appearance for several days.

It is interesting to recall that the growing zygotes underwent somewhat parallel changes in the non-pedunculated epithelial cells lining the stomach. They lost their nuclei, stained more intensely blue and increased in diameter several hundred per cent., but their morphology was very different, perhaps because they were subjected to the digestive ferments of the cells harbouring them; whereas these post-oökinete stages in the salivary gland cells have smooth outlines and are walled off from their unresponsive cytoplasmic environment by a fluid barrier which does not stain.

The next change consists of the appearance of spherical blue staining masses within these large spherules. Such a condition is represented in Fig. 55 of an adult tick 32 days after engorgement and 8 days after moulting. The salivary gland cell which is acting as host to the parasite is included, and again we observe the clear halo separating its cytoplasm from the contained parasite. The subsequent fate of the blue-staining masses indicated that they are to be looked upon as sporoblasts. We were not successful in determining the number developed by each oökinete, but it must be very considerable and may be variable. A parallel phase in the life cycle is represented in the photomicrograph, Fig. 17, of a portion of the salivary glands of an adult female tick 8 days after moulting and 32 days after engorgement as a nymph. In the centre of

the photograph a large oval mass of material is observed which is intensely stained but within which can be distinguished spherical bodies—the sporoblasts. Its surface is characterised by small swellings and is marked off from the cytoplasm of the host cell to the right, below and to the left by a clear halo. Above is to be seen the nucleus of the host cell.

The segmentation of the surface layer and of the deeper parts into sporoblasts, indicated in Fig. 17, proceeds rapidly with the development of a phase which looks sometimes like a mulberry or a "sporont." We have been influenced in our employment of this term through its use by Dennis in his description of the stage following that of the oökinetes in *B. bigemina*. Our sporonts are, however, very different from his and, as will be seen, give rise to sporoblasts which in turn form sporozoites instead of to migratory sporokinetes and subsequently to sporozoites.

The sporonts are structurally the same whether they form in the "clear" faintly staining cells of the salivary glands or in the "dark" cells, which are more numerous and, since they have a greater affinity for stains, form a different background. A young sporont in a clear cell is shown in Fig. 18. It is in the middle of the figure and is recognisable by its surface buds reminiscent of those beginning to form on the parasite represented in Fig. 17. Here almost the entire basophilic mass is broken up into buds (sporoblasts) and looks less dense. In the preparation itself some red-staining material is visible in each bud in addition to the blue exclusively seen in the original of Fig. 17. To the left and below the thickest portion of this sporont is a small part of the nucleus of the cell containing it, which is of the "clear" type and the outlines of which are consequently indistinguishable in the photograph.

It will be recalled that red-staining (nuclear) material disappeared soon after the parasite entered the intestinal epithelial cells, that it was not seen during the growth stages but appeared during oökinete formation only to disappear again soon after the oökinetes invaded the salivary glands. Now it is appearing a second time in the buds of the sporonts. It remains until the cycle of the parasite in the tick is completed.

(*b*) *During the first* 3 *days on susceptible animals.*

This period is of importance because it is known that ticks capable of transmitting the disease do not do so immediately after they begin to feed on susceptible cattle. Nuttall and Hindle (1913) reached the conclusion that "Infected ticks do not produce infection during the first 2 days when feeding on cattle." In order to test the theory that as a pre-requisite it was necessary for ticks to be made infective by the warmth of the animals, these investigators kept some ticks at 37° C. for 3 days after which they were placed on cattle with the interesting result that they also failed to transmit East Coast fever during the first interval of 2 days. In concluding their paper Nuttall and Hindle made the following statement: "By way of a working hypothesis, we assume that the final development of the parasite in the tick, resulting in the latter

becoming infective, only commences after the tick has begun to take blood." More recently, and on the basis of much experience, Walker (1930) has described the situation as follows: "...an infected adult tick transmits East Coast fever only after it has been biting for a period of not less than 60 hours, and is then only infective for a period of 60 hours, so that after the lapse of 120 hours it can no longer transmit the disease."

Counting all the ticks of our four infective series we examined in serial sections a total of ten individuals *1 day* after they were placed on susceptible calves and found three to contain parasites in their salivary glands. All of them were in the stage which we have termed "sporonts." Fig. 19 shows such a sporont in one of these ticks. The halo about the parasite is distinct and its outline is marked by a circle of buds or sporonts which in the preparation appear red or partly red. The central part of this sporont is developed beyond the condition shown in Fig. 18. It is less dense, it is stained a light bluish green and the sporoblasts have moved from it to the periphery. A careful search was made of the digestive tracts of all three ticks possessed of parasites. No blood cells could be detected. The lumina were not sufficiently dilated to indicate the taking up of cell-free plasma or lymph.

After *2 days*, four out of ten ticks examined contained parasites. In three of them sporonts were observed which apparently did not differ from those just described, but in the fourth (an adult female) the sporoblasts were further separated from the parent mass—a change which seemed to be preliminary to the more radical development found after 3 days. In this particular tick no blood cells could be seen in the lumen of the digestive tract, but the lumen was slightly but distinctly dilated, perhaps by the taking in of fluid from the animal on which the tick fed, but of this we could not be certain.

After *3 days* a very striking alteration was discovered in the organisms in all five parasitised ticks of the nine ticks examined, which is well illustrated in Figs. 57 and 58. These figures were drawn from an adult tick of the second nymphal series (Table III) 38 days after engorgement with blood containing parasites and 14 days after moulting. Both figures are limited to the margin of what remains of the sporonts. In the first, the space between the sporont and the cell wall (represented in blue) is clear, while in the second it is filled with a homogeneous-looking green-stained deposit, which in our experience is more commonly encountered in nymphs which engorge on infected blood as larvae than in adults which engorge as nymphs.

Fig. 57 should be compared with Fig. 56, which latter represents a less-developed sporont in a tick before feeding. It will be noted that the red-stained chromatin represented in Fig. 57 is becoming arranged in small masses on the surfaces of the sporoblasts. The differentiation of these chromatin masses is rather further advanced in Fig. 58, though it does illustrate conditions in the same tick. The masses have in many cases broken loose from the buds, and each is seen to be made up of some blue-stained cytoplasm in which a single spherical or cup-shaped nucleus is embedded. These small forms are remarkably uniform

in shape, and we believe them to be the final infective stages, or sporozoites, of the parasite. The sporoblasts on which they develop are generally coloured blue (Fig. 57) by Giemsa's stain after fixation in Bouin's fluid, but they may be red (Fig. 58), or light blue with deep blue (Fig. 62) or red centres (Fig. 63).

While the development of sporozoites was observed in association with the sporoblasts in all of the five ticks examined after 3 days, well-formed sporonts like those illustrated in Figs. 18 and 19 were only seen in one. All of the ticks seemed to have a slightly increased amount of fluid in their digestive tracts. In only one of them, however, could ingested leucocytes be recognised.

(*c*) *From* 4 *to* 18 *days on susceptible animals.*

For convenience this period may be divided into two parts: before engorgement (Figs. 20–25, 60–67), and after engorgement (Figs. 26–28, 68, 69).

It is comparatively early that the sporoblasts are most active in the production of sporozoites and the sporonts are broken up. The condition of the parasite in a tick *4 days* after being placed on calf 5199 is partly illustrated in Figs. 59, 66 and 67. In the last two are represented sporoblasts with the small forms developing peripherally. In the first (Fig. 59) is shown a mass of material composed of the residue of the sporoblasts after the sporozoites have been produced. It will be noticed that the sporozoites surrounding this mass have lost the definite orientation which is so characteristic in respect to active sporoblasts. The morphology of the sporozoites is best shown in this figure (Fig. 59). They are not spherical, but elongate with a single nucleus located toward one end which is generally blunter than the other.

The production of sporozoites is further illustrated in Figs. 60–65, each one representing a single sporoblast in a tick 1 day later (*i.e. 5 days*). These sporoblasts are distinctly larger, as are also the chromatin masses and the developing sporozoites (Fig. 63). The sporoblast shown in Fig. 65 is, like the mass represented in Fig. 59, a residue. It is no longer giving rise to sporozoites and shows unmistakable signs of degeneration. The relationship between the sporoblasts and the sporozoites may be clearly distinguished in the photomicrographs, Figs. 22 and 25, also from a tick allowed to feed for the same period of 5 days.

The disintegration of the sporonts by the spreading apart of the sporoblasts is to be attributed to this tremendous development of sporozoites. The sporonts were found to be the largest, and the sporozoites developed from them to be the most numerous in the case of five out of seventeen female ticks found naturally attached to an animal in a region very heavily infested with East Coast fever. The remaining twelve ticks did not show parasites. Just how long they had been attached we do not know. Some of them had taken in leucocytes but no red blood cells, which, under the physical conditions which have been described, is an indication of their having fed *about 5 days*. In these ticks the parasitised cells were so greatly distended that in photographs taken at the same magnification they could not be included in the same-sized figure (see Figs. 20 and 21). Such cells often have a maximum diameter of 100 microns,

and because the pressure within is so great they are usually more or less globular. Never in our ticks fed on animals reacting to East Coast fever which were later reared and moulted in the laboratory was the infestation with parasites so intense, though the properties of the individual organisms seemed identical.

In Fig. 20 a section through one of these immense sporonts is shown. The peripheral sporoblasts are clearly outlined and are stained red in the original preparation. Minute sporozoites are present in abundance both in the centre of the sporont and about its circumference, having been formed, as described, from the sporoblasts. In Fig. 21 the original sporont is broken up by the pressure of the sporozoites into a number of daughter sporonts. One of these especially, located in the centre of the figure, recalls the smaller and younger sporonts illustrated in Figs. 18 and 19. Some of the sporozoites are being discharged into the lumen of the acinus to the left, but this is not well shown in the photomicrograph.

Smaller masses of parasites were discovered in ticks of our standard series *5 days* after they were placed on susceptible animals. They are illustrated in the photomicrographs, Figs. 22–25. The formation of sporozoites by the sporoblasts indicated in Fig. 22 has already been referred to. Fig. 23 is of value because in it are included parts of the cytoplasm of two parasitised salivary gland cells. In the one in the upper right-hand part of the figure are many sporoblasts of variable size with sporozoites in association. In the one to the left, which we interpret as a rather more advanced stage, the sporozoites are more numerous and still smaller in size. They are, moreover, very densely packed together except in the vicinity of a few inactive sporoblasts which have fulfilled their function of producing the sporozoites.

Fig. 24 shows a mass of sporozoites which seems ready for discharge into the lumen of the acinus to the right. The parasites in Fig. 25 are all of the very small variety with the exception of a single sporoblast at the surface of which a few sporozoites can be seen. To the right of this sporoblast is the degenerate nucleus of the parasitised salivary gland cell. Such nuclei commonly persist until after the cells are completely freed of parasites. These tiny sporozoites are typically grouped together in the centre of the cell surrounded by a halo of cytoplasm devoid of parasites.

We now pass to a discussion of our findings in ticks which have ingested much blood. Fig. 27 illustrates a portion of an acinus with a cell containing many parasites in a nymph 39 days after engorgement as a larva, 15 days after moulting and *6 days* after being placed on calf 5840. Many sporoblasts are visible with sporozoites in close association. Fig. 28 is from another tick *8 days* after attachment to the same calf. It is of particular interest, because it shows the desquamation into the lumen of the acinus of a large mass of parasites of irregular size and variable staining reaction which seems to be on the road to degeneration.

Although one would expect the life cycle of the parasite in the tick to be

terminated in this way and about this time, some remain much longer. Figs. 68 and 69 were drawn from a tick of the larval series (Table I) 43 days after engorgement with blood from a case of East Coast fever, 19 days after moulting to a nymph, and *11 days* after being placed on calf 5840. Fig. 68 shows two buds and many small forms of the parasite. The sporoblast to the left has the appearance of being inactive because the sporozoites are not regularly arranged about it, whereas there is definite orientation of sporozoites in respect to the sporoblast on the right. Fig. 69 shows the discharge of sporozoites into the lumen of the acinus (*i.e.* toward the left). It is possible, therefore, that both manufacture of sporozoites and their discharge into the lumen may persist in engorged ticks, though passage into the lumen of a salivary gland acinus is not by any means synonymous with passage along the salivary ducts into the animal on which the ticks have almost engorged.

(9) *Experimental proof of the infectivity of ticks containing parasites in their salivary glands.*

Parallel material was collected as follows from the feeding ticks and the calves they fed upon.

(*a*) *The ticks and the skin fed upon.* With a sharp razor wedge-shaped pieces of skin, including some subcutaneous tissue and the attached ticks, were removed. The ticks were separated from the skin by cutting through their mouth-parts with scissors. Numbers were given to the specimens so that it was possible at any time to determine which tick had been feeding on any given piece of skin. The chitin was dissected from the ticks in the manner already described, and both ticks and skin were fixed separately in Bouin's fluid.

(*b*) *Pieces of lymph glands.* Under chloroform anaesthesia small pieces of the superficial lymph glands were removed with aseptic precautions and fixed for sections in (i) Bouin's fluid and (ii) the sublimate, alcohol acetic acid mixture. The object of using the latter fixative was to secure material suitable for applying Feulgen's reaction for thymonucleic acid.

(*c*) *Smears of lymph glands and blood.* The gland smears were made with the fluid which entered a hypodermic needle plunged into one of the superficial lymph glands, and the blood smears with blood taken from the tip of the tail or the margin of one of the ears.

(*d*) *Pieces of the principal organs.* These were collected when the calves were killed 20, 8, 15 and 16 days, respectively, after infestation with ticks.

The results of the examination of the ticks have been included in Tables I–IV. The specimens from the calves will, with these special ticks, form the basis of the second half of this contribution dealing with "The behaviour of the parasite and the development of distinctive lesions in susceptible animals," as mentioned in the introduction. It is sufficient to remark here that:

Calf 5840 (Table I) showed typical Koch's bodies in both gland and blood smears beginning on the thirteenth day after infestation with the ticks.

Calf 5199 (Table II) exhibited parasites in red blood cells 2, 4, 5, 6 and 7

days after infestation with ticks. These parasites were determined as *Theileria mutans*, though they had appeared altogether too promptly to have been transmitted by our standard series of ticks. On the eighth day this animal died while we were attempting, under chloroform anaesthesia, to remove a piece of lymph gland for histological study. No Koch's bodies were observed in either blood or gland smears, but from our findings with the other three calves it seems likely that the time for their development may not have been sufficient.

Calf 5869 (Table III) showed Koch's bodies in gland smears beginning 9 days after infestation with ticks and in blood smears 4 days later. It developed a characteristic attack of East Coast fever.

Calf 5838 (Table IV) acquired the disease more slowly. Koch's bodies were observed in gland smears 15 and 16 days after infestation, but they were not seen in the blood smears. Clinically the case showed, like Nos. 5199 and 5869, all the classical symptoms of East Coast fever, but to a slight degree.

It is evident, therefore, that three of our four series of ticks which contained parasites in their salivary glands were capable of transmitting East Coast fever to susceptible animals. It is probable that the other series (Table II), which possessed parasites in equal abundance, would also have transmitted the disease if the animal infested had not died so soon.

V. Discussion.

(1) *Outline of the life cycle of the parasite.*

As a preliminary to our discussion we wish to repeat with the aid of Fig. I the sequence of events in the tick omitting many of the details contained in the preceding pages.

(1) After the tick takes in blood from a case of East Coast fever the ingested red blood cells can be seen to contain parasites of many shapes and sizes. Some are small and possess a nucleus which stains very deeply and which volumetrically is about as large as the cytoplasm. Others are much larger and contain nuclei which colour less intensely and are embedded in a relatively much greater amount of cytoplasm. Both show indications of division within the corpuscles, but this division is not often repeated. The small ones may be males and the large ones females, but in the absence of evidence no conclusion is warranted.

(2) Within the gut of the tick and after escape from the corpuscles two similar types of parasite can be distinguished, large and small. There is a marked tendency for each to be associated in clumps.

(3) The large and small parasites become applied to the surface of epithelial cells lining the gut and fertilisation probably takes place, but we have not discovered convincing evidence of it.

(4) The parasites enter these cells and the small forms rapidly disappear. The large forms grow and give rise to a stage without distinct nuclei and not previously seen, which we refer to as a zygote.

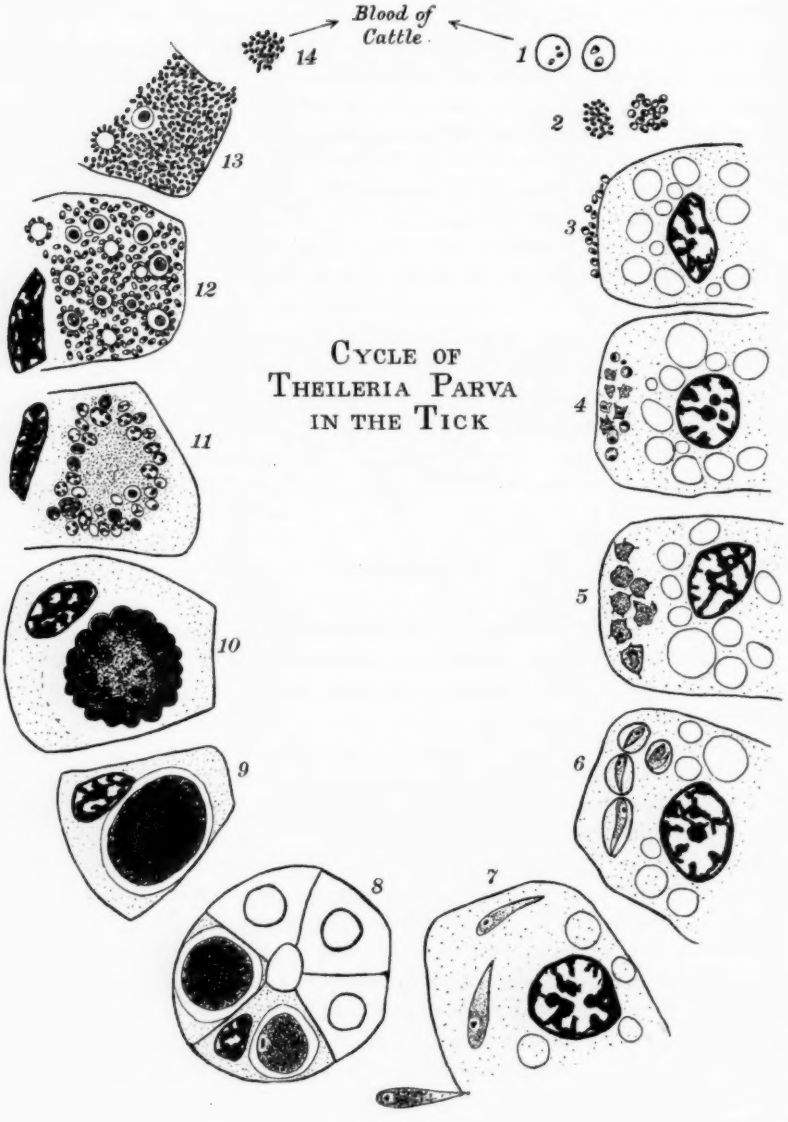
Blood of Cattle
1
2
3
4
5
6
7
8
9
10
11
12
13
14
CYCLE OF THEILERIA PARVA IN THE TICK

Fig. I.

(5) These zygotes increase greatly in size. Nuclei reappear within them and some of them exhibit a central concentration of material and an accentuation of their limiting membranes.

(6) The central concentration becomes more marked and gradually assumes the form of a large elongated nucleated organism which we call an "oökinete."

(7) By rupture of the distended limiting membrane of the original zygotes the oökinetes escape into the cytoplasm of the epithelial cells lining the gut. They then make their way into the body cavity, where they may be found in close association with the salivary glands.

(8) The oökinetes enter the cells of the salivary glands where they round up, but may at first be recognised by the characteristic blue staining of their cytoplasm and their sharply defined nuclei. The cytoplasm of the salivary gland cell in contact with them does not take the stain in the usual way, so that they seem to be surrounded by a kind of halo. The nucleus soon disappears and the parasite increases in size and becomes coloured more intensely but retains its halo.

(9) There is further growth and the cell containing the parasite becomes noticeably distended. The halo persists.

(10) About the periphery of the mass buds appear; its central part colours less intensely with Giemsa's stain, and the clear outlines of the halo are lost. We designate this stage a "sporont," and regard the peripheral buds as "sporoblasts" in process of differentiation.

(11) The sporoblasts become much more distinct and exhibit within their interior irregular masses of chromatin. At the same time the central part of the sporont, which does not contain sporoblasts, loses progressively its affinity for stains.

(12) The sporoblasts develop rapidly. They possess a variable amount of chromatin in their interior. Their peripheral substance gives rise to sporozoites which are oriented rapidly in reference to them. Many of these sporozoites become detached from the parent sporoblasts.

(13) The size of the sporozoites is reduced about one-half. They are very small and consist of a slightly elongated mass of blue-staining cytoplasm, in one extremity of which is situated a deeply red-staining nucleus. A few of the sporoblasts remain. Some of them are still active in the formation of sporozoites, but the majority have lost this function and no longer have sporozoites regularly applied to their surfaces.

(14) The sporozoites, which look very much like the small forms observed both in the red blood cells (1) and free in the gut of the tick (2), are discharged into the lumen of a salivary gland acinus, whence some of them are presumably passed into the animal on which the tick is feeding and to which it is transmitting East Coast fever.

(2) *Comparison of our findings with those of other investigators.*

It is interesting to compare our observations with those already described by other investigators relative to the life cycle of piroplasms in ticks, although it is not to be expected that different species will behave in precisely the same way. Particularly is a difference to be expected between members of the genus *Babesia*, in which the parasites are known to be passed hereditarily from one generation to the next in the eggs, and members of the genus *Theileria*, in which hereditary transmission of parasites has not been observed experimentally. It should be remembered also that *Theileria parva* is one of the smallest of the piroplasms, and that for this reason morphological changes quite apparent in the others may be entirely suppressed or so slight as to escape detection. Previously published reports relate to:

Babesia bigemina (Koch, 1906; Dschunkowsky and Luhs, 1909, 1909*a*; Dennis, 1931).

Babesia canis (Christophers, 1907, 1912).

Theileria parva (Koch, 1906; Gonder, 1910, 1911, 1911*a*; Cowdry and Ham, 1930).

Piroplasma equi (Marzinowski and Bielitzer, 1909).

Unfortunately we have not had an opportunity to consult the original contributions of Dschunkowsky and Luhs. We have had to rely upon the reviews in the *Bullein de l'Institut Pasteur* and the comments of other investigators.

(*a*) *Distinction between male and female forms.*

We have referred to small and large forms of the parasite which may be males and females, but concerning which distinction we cannot speak with any assurance. We hope at a later date to search for further evidence. Gonder has, however, described micro- and macro-gametocytes, gametes and even the process of copulation. We have observed and figured all of the morphological forms of the parasite which he has illustrated, with the exception of his oökinetes, but we do not feel justified in subscribing to his interpretation.

In respect to *B. canis*, Christophers (1907) remarks that "like myself Nuttall and Graham-Smith have failed to detect any forms of the parasite which there seems reason to regard as gametes." In their description of *P. equi*, Marzinowski and Bielitzer say that in general two types may be distinguished at about the second day, one with dark blue coarsely granular protoplasm and small nucleus, generally oval and worm-like in shape, and another having a somewhat smaller nucleus and lightly stained protoplasm; but they do not hazard any opinion as to which is male and which is female. Dennis makes the positive statement that in *B. bigemina* the male and female gametes are indistinguishable, for which reason he calls them "isogametes."

(*b*) *Forms with spiky pseudopodia.*

Our investigations have not revealed any close counterparts of the large forms with spiky pseudopodia described in the gut of the tick by Koch (1906)

as developmental stages of *B. bigemina*. Judging from his excellent coloured lithograph, the length of these pseudopodia is often five or six times the diameter of the rather well-defined and nucleated body of the parasite. Similar, but much smaller, forms have been reported by him as stages in the life cycle of *T. parva* (see his Figs. 50–61). It is important not to confuse such spiky pseudopodia with the "flagella-like processes" described about the same time by Nuttall and Graham-Smith (1906) in their monograph on canine piroplasmosis (Diagram 8). The latter are not straight but undulating, and are never so long. Christophers (1907) discovered stages of *B. canis* with spiky pseudopodia, like those of Koch. Judging from his illustration (Pl. I, fig. 48) they were not nearly so conspicuous as the forms of Koch. Parasites having similar straight ray-like processes were again reported by Dschunkowsky and Luhs in *B. bigemina* and by Marzinowski and Bielitzer in *P. equi*; but here again the individual parasites represented by the latter authors (Fig. 10) only faintly resembled those of Koch. Analogous forms are not reported by Dennis who also studied *B. bigemina*. In our preparations of *T. parva* the nearest approach to a parasite possessing spiky pseudopodia is represented in Fig. 31, *v*. The investigators mentioned were all careful observers, and we must accept the actual occurrence of the forms with spiky pseudopodia thus repeatedly described. Their interpretation is, however, another matter in which we have to estimate the possibilities.

There is a good deal of scepticism concerning them. Christophers does not regard them as an essential stage in the development of the parasite—an opinion shared by Wenyon (1926). Marzinowski and Bielitzer do not attribute any diagnostic significance to them. They may be degeneration forms of some animal or plant parasite, not necessarily the one under investigation at the time, because ticks are often abundantly parasitised. The only known parasite which is likely to occur in the large number of species of ticks involved is some kind of symbiont (see Cowdry, 1925), but we have studied many symbionts without seeing star-shaped forms with pseudopodia like those reported. Neither have we detected normal nor degenerating cells belonging to the ticks which could be confused with them.

It seems more likely that these strange forms are modifications of the parasites under observation attributable to the technique used. They have been seen in fresh preparations (Marzinowski and Bielitzer) as well as in stained smears. The one consistent observation by all who have reported them is that they occur in the earliest stages of the cycle of the parasites in ticks. Thus, Marzinowski and Bielitzer discovered them on the first and less frequently on the second day. An examination of Koch's Figs. 5–16 shows a kind of gradation from parasites with small or no spikes to others in which the spikes are highly developed. A more definite seriation is indicated in Kleine's illustrations of changes in *P. canis* in cultures of infected blood plus physiological salt solution. Nuttall and Graham-Smith (1908) have likewise encountered in cultures of *B. canis* certain large forms with long radiating processes—similar

to those described by Kleine and Koch. But in a later attempt (1909) to cultivate *T. parva* they make no mention of them. In cultures of any kind the conditions are unusual and approximate only to those which obtain *in vivo*. Since we have, ourselves, seen only the slightest indications of such processes in smears and have never observed forms which really resemble those figured by Koch, Kleine, Nuttall and Graham-Smith and others, we cannot advance any definite opinion as to their nature; but the hypothesis which appears to be most consistent with the observations described is that we are faced by distortions of the parasites in a very early stage of their development caused by osmotic and other factors in the technique used.

(*c*) *Conjugation.*

According to Marzinowski and Bielitzer the forms which we have mentioned with spiky pseudopodia are less frequent on the second day. On the second and third days they report forms which point to the conjugation of two parasites but do not describe them. Gonder (1911*a*) presents drawings (Figs. 34–37) and a photomicrograph (No. 2) which he claims to illustrate this process. We have seen and illustrated similar appearances (Fig. 31, *a*, *d*, *r* and *s*), but they do not prove conjugation. In his very recent note Dennis is not very specific on this point. In describing *B. bigemina* he simply says: "Fertilisation apparently takes place by the isogametes becoming associated first at the blunt anterior end, and then fusing." From the sequence in which he gives his results it would appear that this phenomenon occurs in the lumen of the gut.

(*d*) *Intra-epithelial growth stages of zygotes.*

This stage in the development of piroplasms has not been specifically described by other workers. It is natural for Koch and Christophers to have omitted reference to it because their investigations were not supported by serial sections which alone would give information as to the intra-epithelial position of the parasites. Nevertheless, in one of his figures Christophers shows a phase of *B. canis* which looks like some of our intra-epithelial stages of *T. parva*. Compare for instance our Fig. 40, *a*, *b*, *g* and *h* and Fig. 41, *e* and *k* depicting the appearance in sections and Figs. 8, 9 and 10 of smears. In both the outlines of the parasites are rather irregular, the cytoplasm stains but faintly and the nuclei are spherical and small in comparison with the size of the cytoplasm. Similarity of the same kind obtains between our figures just mentioned and Fig. 15 of *P. equi* by Marzinowski and Bielitzer. The parasites represented in our figures are in a stage preliminary to oökinete formation. An exact correspondence between our findings and those of Christophers is not to be expected because he was dealing with *B. canis*, which belongs to a different genus and is much larger than *T. parva*.

Although Gonder did employ sections in his study of *T. parva* he did not report any parasites resembling these intra-epithelial growth stages of the zygotes. The only reference which Dennis makes to growth of *B. bigemina* within the intestinal epithelium is as follows: "The oökinetes penetrate the

tissues of the thin gut wall where they round up, grow and form *sporonts* ranging from 5·0 to 12·0 microns in diameter, or they may pass completely through the gut wall and enter the ovary where they invade the ova." It would appear from this statement that a period of development within the intestinal epithelial cells of the gut is not so essential a stage in the cycle of *B. bigemina* as in the case of *T. parva*. In our four series, *T. parva* underwent development in this situation over periods of 6–27, 9–34, 6–28 and 7–23 days after engorgement.

(*e*) *Development and migration of oökinetes.*

In the formation of his "club-shaped bodies," which seem to correspond to our oökinetes, Christophers describes in *B. canis* the segmentation of large spherical masses (presumably resulting from conjugation) as marked by the appearance on their surface of a Y-shaped "achromatic" line. These achromatic lines are illustrated in his 1907 paper (Pl. I, fig. 52, and diagrammatically five years later (1912), Diagram 2). In this later paper he cites the work of Marzinowski and Bielitzer on *P. equi* in support of his own. "Marzinowski and Bielitzer's Fig. 18, Pl. VI, shows what is evidently a club-shaped body in process of formation as described by me on pp. 21 and 33. The upper part of fig. 19 also shows the achromatic line to which I have referred, the same line (indicating the formation of the tail of the club-shaped body) can be seen in several groups of parasites shown in fig. 17."

A detailed examination of the figures of Christophers and Marzinowski and Bielitzer does not lead us to the same interpretation of the bodies showing achromatic lines. We have seen somewhat similar forms in our study of *T. parva* but instead of being stages in the life cycle of the parasite they were concretions from the rectal sac which in the drying of the smears became cracked on the surface giving rise to lines which were achromatic in the sense that they did not take the stain. These appearances were noted in smears of "clean" as well as infected ticks but not in sections. Other investigators, who have worked on the life cycle of piroplasms in ticks, have not to the best of our knowledge reported as essential developmental stages forms with achromatic lines resembling those of Christophers.

According to Christophers (1912): "The club-shaped forms are the most easily detected stage in the developmental cycle and can generally be found without difficulty about the fourth or fifth day in ticks which have been fed on heavily infected dogs. For their detection fresh preparations are made of the ovary, oviducts, etc., and the field examined under a relatively low power for the actively motile small leech-like immature club-shaped bodies or the more rigid and less active maturer forms." Marzinowski and Bielitzer do not state precisely the time of development of oökinetes. They simply remark that conjugation forms appear about the second and third day, and that as a result of this conjugation new forms are built up which look like small worms and which correspond to the descriptions of Koch and Christophers. Neither is the appearance of oökinetes definitely dated in Koch's original paper, but the impli-

cation is that they are to be seen soon after the third day which is very much earlier than we found the oökinetes of *T. parva* which did not appear until about the time of moulting 25, 23 and 15 days after the ingestion of blood containing parasites (see Tables II–IV). Dennis does not mention the time of development of the oökinetes of *B. bigemina*.

Koch and Marzinowski and Bielitzer fail to give any information as to the site in the tick of the formation of oökinetes. Christophers would look for them in the ovaries, oviducts, etc. Gonder does not mention in any of his papers where the forms which he calls the oökinetes of *T. parva* are formed. According to Dennis, those of *B. bigemina* are formed in the lumen of the gut, which is in sharp contrast with the development of the oökinetes of *T. parva* which we have found to occur only within the epithelial cells lining the gut.

The properties of the oökinetes of these several parasites are not uniform. The oökinetes described for *T. parva* by Gonder are very different from ours, although he mentions their appearance "on the eve of moulting" which is almost exactly the time that we have reported their development in our series. A comparison of his Figs. 48 and 40 (1911*a*) with our Figs. 13, 14 and 49 shows that his oökinetes: (1) taper about equally at both extremities whereas ours are blunter at the anterior end, (2) possess in proportion to the cytoplasm a very much larger nucleus and (3) that the nucleus is placed about equidistant from the extremities, while in our oökinetes it is invariably found in the anterior part of the parasite. What stage of the life cycle of *T. parva*, if any, Gonder was dealing with we do not know. The oökinetes described by Koch in *B. bigemina*, by Christophers (1907) in *B. canis*, and by Marzinowski and Bielitzer in *P. equi*, resemble ours much more closely. Nevertheless, we think that a detailed comparison would bring to light slight but definite differences, for the parent parasites are themselves different. We have not observed in *T. parva* anything suggestive of the fission of oökinetes as indicated for *B. canis* by Christophers in his Pl. II, figs. 12 and 15. The oökinetes of *P. equi*, as illustrated in Figs. 20–22 of Marzinowski and Bielitzer, are much straighter than the oökinetes of *T. parva* shown in our Figs. 13, 14 and 49. They also exhibit a more abruptly rounded anterior extremity and a proportionally larger nucleus which is not so characteristically situated in the anterior position of the parasite. The oökinetes of *T. parva*, as far as we have been able to discover, do not exhibit the very active movements attributed to those of *B. canis* by Christophers and of *P. equi* by Marzinowski and Bielitzer, but instead glide slowly about in an effortless manner. Until Dennis publishes a detailed account it is not possible to compare profitably the oökinetes which he found of *B. bigemina* with those of *T. parva*.

From their large size and striking appearance it is but natural that the oökinetes should have been reported on several occasions as we have just noted. There is by contrast very little information contained in the literature as to their fate. Christophers was able to trace the oökinetes to the ovaries, as one would anticipate, because *B. canis* is inherited via the eggs. In the case of

T. parva, which is not inherited, a migration to the ovaries is not to be expected, and we have found that it does not occur. Gonder was unable to follow the structure which he termed oökinetes, but assumed that they entered the salivary glands. As far as we have been able to ascertain the immediate changes which the oökinetes of *T. parva* undergo in this situation have not been reported before as in the case of any kind of piroplasm. In *B. bigemina*, Dennis found that the oökinetes either come to rest in the wall of the gut and form sporonts there or else "pass completely through the gut wall and enter the ovary where they invade the ova" and give rise to sporonts. The attraction of the ovary for the oökinetes of *B. bigemina* is therefore not quite so exclusive as is that of the salivary glands for the oökinetes of *T. parva*.

(*f*) *Formation of sporonts, sporoblasts and sporozoites.*

No stages corresponding with our sporonts and sporoblasts of *T. parva* were described by Gonder. The "zygotes" of *B. canis* noted by Christophers may constitute a stage parallel with our sporonts. Wenyon (1926) has estimated the evidence presented by Christophers as follows: "The formation of club-shaped bodies and the development of zygotes, as Christophers terms the growing forms developed from them, were studied in nymphs fed on infected dogs. The sporoblasts become distributed amongst the various developing embryonic tissues, some of which are undoubtedly destined to become the salivary glands of the adults. The developmental process was studied in dried smears, and it is evident that further investigations with more reliable technique are required. It seems quite possible that some of the stages described as zygotes are actually tissue cells of the tick."

The preliminary statement of Dennis would seem to forecast the clearing up of the situation as far as *B. bigemina* is concerned, and his observations are almost the only ones with which ours can be compared, for it is doubtful whether Koch, Christophers, Gonder, Marzinowski and Bielitzer found any intermediate stages between the oökinetes and the sporozoites, though it seems possible that all of them, except Koch, observed the latter.

Dennis found that the sporonts of *B. bigemina* divide "to form multinucleate sporoblasts." These sporoblasts are amoeboid and actively motile, and migrate throughout the embryonic tissues of the developing tick. Because of their migratory nature these somatelles are called sporokinetes. The sporokinetes are very phomorphic and have from four to about thirty-two nuclei. During the ontogeny of the tick the sporokinetes may come to infest almost any tissue of the embryo. Since much of the anterior embryonic cell mass of the tick is destined to contribute to the salivary glands, some of the alveoli come to be occupied by sporokinetes. As the incubation period of the tick draws to a close, some of the sporokinetes undergo multiple fission of "sporogony" to form sporozoites; others may not form sporozoites until after the tick has hatched. The sporozoites are miniature trophozoites and may also multiply, doing so by the "budding" process which is characteristic of the trophozoite.

The sporozoites are inoculated into the blood stream of the bovine host in the saliva of the feeding seed tick, and soon set up an active infection of the erythrocytes of the host.

Our observations on *T. parva* show that sporonts are only developed in the salivary glands. The sporoblasts which are formed from them remain in the parasitised salivary gland cells, never becoming migratory as in *B. bigemina*, so that there is no stage in the life cycle of *T. parva* corresponding to the sporokinetes of *B. bigemina*. The sporozoites formed from the widely distributed sporokinetes of *B. bigemina* are probably to be found in almost all the tissues of the offspring of female ticks which engorged with blood containing the parasites. It is only apparently by chance that those descended from sporokinetes in the anterior embryonic cell mass come to occupy a position in the salivary glands. The sporozoites of *T. parva* are, on the contrary, strictly limited to the salivary glands. The difference is perhaps simply the consequence of hereditary transmission of the former, while the latter remains in the tick only through one period of moulting.

It is difficult to make a comparison of the time when the sporozoites of the two parasites become mature and are ready for transmission to the next susceptible host. From the account of Dennis it would seem, however, that the sporozoites of *B. bigemina* are formed over a considerable period which begins before the ticks are hatched. This point we wish to emphasise, because it is obviously also before the ticks have a chance to feed. The sporozoites of *T. parva* are first developed from the sporoblasts later, about the third day after the ticks are placed on the animals next in series.

VI. Summary.

1. In addition to the parasite of East Coast fever three other organisms were observed. The first of these is a "symbiont" and was found in the Malpighian tubules and ovaries of all the ticks examined; the second, a protozoan, was observed in about 50 per cent. of our ticks in the process of multiplication in the epithelial cells of the gut and in the large phagocytic cells of the body cavity; the third, from its very active movements, probably a ciliate, was seen only in dark field preparations of a few ticks recently engorged. Once recognised these organisms were always easily distinguished from *Theileria parva*. We were unable to confirm the assumption of Nuttall and Hindle (1913) that practically 100 per cent. of ticks fed on blood containing parasites become infective and are thus capable of transmitting the disease. Approximately accurate figures on the survival of ingested parasites can only be secured from sections. Of the 290 ticks of our standard series examined in very complete serial sections, parasites were found in 178, or 60·1 per cent. In the first series of infective nymphae, in which we kept records of sex, 33 per cent. of the ticks containing parasites were females and 66 per cent. were males.

2. In the erythrocytes of the animals suffering from East Coast fever on

which the ticks engorged large and small parasites were distinguishable (Figs. 29 and 30 and Fig. I, p. 32). Both showed signs of division.

3. Intra-erythrocytic parasites were observed in the gut of the ticks only up to the sixth day after engorgement (Table V). Liberation of parasites takes place at a different rate in different ticks.

4. Parasites were seen free in the lumen of the gut from the time of engorgement up to 10, 13, 14 and 8 days, as indicated in Tables I–IV. Beginning about 2 days after engorgement and until they disappear from the gut there is a marked tendency for the parasites to be arranged in clumps, the small ones together and the large ones together (see Fig. I at 2). But the small and large parasites are sometimes scattered indiscriminately. Both types tend to accumulate on the surfaces of the epithelial cells lining the gut (Fig. I at 3).

5. Many parasites are taken up by epithelial cells which stretch further into the lumen than their neighbours and are called "pedunculated." They are accompanied by lymphocytes, leucocytes, platelets and a variety of foreign materials. Parasites were observed in this situation from engorgement to 14 days (Table I), from the second to thirteenth day after engorgement (Table II), from the sixth to the twenty-first day after engorgement (Table III), and from the first to the eighth day after engorgement (Table IV). The examinations listed in Table III began on the sixth day. The fact that parasites were not seen earlier in this series in the pedunculated cells does not mean that they were absent. On each day during these rather long periods large and small parasites were observed which stained brilliantly and some of which appeared to be dividing. Other individuals of both types stained but feebly and from their irregular outlines were clearly degenerating. How long individual parasites remained could not be determined. Table I shows their presence in pedunculated epithelial cells 4 days after disappearance from the lumen, and Table III, 5 days after. But in the ticks recorded in Tables II and IV they disappeared from the pedunculated cells on the same day that they could no longer be seen in the lumen.

6. It is the parasites which enter non-pedunculated epithelial cells which carry on the life cycle. They enter in smaller numbers and more slowly. Tables I, II and IV record their appearance in this commoner type of epithelial cells just 7 days after they were first noted in the pedunculated cells. They remain intra-epithelial for as long as 20 days (Table I), 25 days (Table II), 22 days (Table III), and 16 days (Table IV). As a rule they are not accompanied by leucocytes and different sorts of debris. The small forms soon disappear. Large forms like those seen in the lumen are at first present in considerable numbers and give rise to a parasite distinctly different from any previously seen, which we call a "zygote," although we have been unable to demonstrate the process of fertilisation. These zygotes have rather irregular outlines, stain blue by Giemsa's method and are for a time without red-staining nuclei. In this condition they are represented in Figs. 1–5, 40 and 41 (*a–d*); also in Fig. I at 4 and 5. In this phase of the zygote the parasites have to resist the

digestive action of the cell containing them and many succumb, but some grow; their diameter increasing 300 or 400 per cent. (compare Figs. 1 and 5).

The surviving ones begin to exhibit once more characteristically red-staining nuclei about 18 days after engorgement and at approximately the time of moulting (Figs. 6, 41, *e–h*). Coincident with this change their outlines become smooth and they are evidently less susceptible to digestion. Oökinetes develop very rapidly within their interior (Figs. 42–49 and Fig. I at 6). All stages in the formation of oökinetes from blue-staining zygotes to others which are fully differentiated and have escaped from the epithelial cells of the gut into the body cavity are frequently seen in a single tick. Oökinetes were observed from the first to the eighth day after moulting in sections and to the ninth day in smears (Table II), from the first day before moulting to the eleventh day after (Table III), and from the second day before to the ninth day after (Table IV). They are illustrated in Figs. 7, 11–14 and 49.

7. The oökinetes begin to enter the salivary glands about the time of moulting. Our failure to detect them in this situation in our larval series (Table I) until 11 days after moulting is a negative observation, possibly due to technical difficulties, and of little significance compared with their definite discovery in the salivary glands 1 day after moulting (Table II), 1 day before moulting (Table III), and 1 day after moulting (Table IV). But this entry is probably not restricted to the days mentioned, for typical oökinetes were observed 9 days (Table II), 12 days (Table III) and 10 days (Table IV) after they were first noted in the salivary glands. It is therefore probable that successive crops of oökinetes enter the salivary glands over a relatively long period and that this explains the discovery of parasites in different stages of development in the salivary glands of individual ticks. Soon after penetration they round up but can be recognised at least until 5 days after moulting by their intensely blue-staining cytoplasm and red-staining nuclei (Figs. 15, 50, 51).

The next stage, which we refer to as a beginning "sporont," is represented in Figs. 16, 52, 54 and to the right in Fig. I at 8. The nucleus of the oökinete is lost, but the parasite can be identified within the salivary gland cells by its intense deep blue colour when stained by Giemsa's method, by the clear halo about it, and by its relatively large size. The salivary gland cell containing it is usually considerably distended. This condition persists for a variable time which we estimate at about 3 days. Approximately 8 days after moulting buds appear on the surface of the sporont (Figs. 17 and 53) and spherical masses within its interior (Fig. 55) which constitute the "sporoblasts." At the same time the central part of the parasite stains less intensely and the halo about it becomes barely distinguishable (Fig. I at 10). A little later on the central part stains still less strongly and the sporoblasts become proportionally more clearly defined (Fig. 18). Red-staining chromatin reappears in irregular masses in the sporoblasts (Fig. I at 11). This, in our experience, is as far as the parasite develops before the ticks containing it are placed on animals with a view to

transmitting East Coast fever. During the first 3 days of feeding on susceptible animals the parasites were examined in great detail.

After *1 day* a total of ten ticks were studied in serial sections, of which three contained parasites in their salivary glands. All of them were in the sporont stage. The central area of the sporont shown in Fig. 19 is developed beyond the condition illustrated in Fig. 18. It is less dense, it is stained a light bluish green and the sporoblasts have moved from it to the periphery. A careful search was made of the digestive tracts of all three ticks. No blood cells could be detected. The lumina were not sufficiently dilated to suggest the intake of cell-free plasma or lymph.

After *2 days* four out of ten ticks examined contained parasites. In three of them sporonts were observed which apparently did not differ from those just described; but in the fourth (an adult female) the sporoblasts were further separated from the parent mass—a change which seemed to be preliminary to the more radical development noted the next day. In this particular tick no blood cells could be detected in the lumen of the digestive tract, which, however, was slightly but distinctly dilated perhaps by fluid from the animal on which the tick fed.

After *3 days* a striking alteration occurred in the organisms in all five of nine ticks parasitised as illustrated in Figs. 57 and 58. The red-stained chromatin represented in Fig. 57 which before (Fig. 56) was distributed irregularly is becoming arranged in small masses at the surfaces of the sporoblasts. The differentiation of these chromatin masses is still further advanced in Fig. 58, though it illustrates conditions in the same tick. The masses have in many cases broken loose from the sporoblasts, and each is seen to be made up of some blue-stained cytoplasm in which a single spherical or cup-shaped nucleus is embedded. These are the sporozoites. While this development of sporozoites in association with the sporoblasts was observed in all of the five ticks well-formed sporonts like those illustrated in Figs. 18 and 19 were only seen in one. All of the ticks seemed to contain a slightly increased amount of fluid in their digestive tracts, but in only one could ingested leucocytes be recognised.

The period from 4 to 18 days is divisible into two parts: before engorgement (Figs. 20–25, 60–67) and after engorgement (Figs. 26–28, 68, 69).

Before engorgement the maximum production of sporozoites takes place. At *4 days* the process is going on with extreme rapidity. Figs. 66 and 67 show two active sporoblasts with sporozoites radially arranged about their periphery. The morphology of the sporozoites on the fifth day is well shown in Fig. 59. When first formed they are somewhat larger than when later on they are ready for discharge. This difference is illustrated in Fig. 23 and in Fig. I (compare stages 12 and 13).

At *5 days* larger sporoblasts were noted (Figs. 60–65), and the sporonts are broken up by the spreading apart of the sporoblasts through the tremendous development of sporozoites (Figs. 20–22). Fig. 24 represents a mass of sporozoites apparently ready for discharge into the lumen of the acinus on the right.

The maximum number of salivary gland cells in any tick containing parasites at any stage in the cycle is about 50 per cent. Such an infestation is, however, very exceptional, occurring in only four or five of the fifty-nine ticks of our standard series possessing parasites in this location. The usual infestation is about 8 per cent. In some ticks, however, parasites are only to be found in one or two cells after long search; that is to say, in a percentage of, say, 0·1 per cent. But when the infestation is so small the individual cells involved may be charged with parasites just as heavily. By far the heaviest cellular charge with parasites, though not the highest percentage infestation, was discovered in five out of seventeen female ticks found naturally attached to an animal in a region in which East Coast fever was very common. The degree of infestation probably depends upon the number of oökinetes which settle in the salivary glands and the individual cellular charge upon adverse or favourable conditions in the salivary glands to the development of parasites.

After engorgement both the percentage infestation and the cellular charge are apparently reduced. The conditions 6, 8, and 11 days after attachment are illustrated in Figs. 27, 28 and 68 and 69. Many of the parasites are obviously degenerating, and the whole mass of parasites may be sloughed off from the cell into the lumen of the acinus (Fig. 29). The last parasites were seen *18 days* after the tick containing them was placed on a susceptible animal (Table IV), which is long past the time of probable transmission of the disease. Our experiments do not show how much longer they persist and it is likely that they are soon destroyed, because it has been proved experimentally by other investigators that the ticks are cleansed by feeding.

8. Three of our four series of ticks, which contained parasites in their salivary glands, which were fed on susceptible animals, produced typical cases of East Coast fever. It is probable that the other series (Table II), which possessed parasites in equal abundance, would also have transmitted the disease if the animal infested had not died so soon under chloroform anaesthesia as we were removing a lymph gland for study.

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EXPLANATION OF PLATES I TO VII.

All the photomicrographs reproduced in Plates I–V were taken with a 2 mm. Zeiss apochromatic objective, ocular and camera bellows extension sufficient to give a magnification of exactly 1400 diameters.

PLATE I.

Fig. 1. To the right, and to the upper and lower left, are the free margins of epithelial cells lining the intestinal lumen. In the lumen are a few scattered, deeply stained, spherical piroplasms, and in the upper part of the figure a lymphocyte with large intensely stained nucleus slightly elongated from left to right. Within the epithelial cells below the lymphocyte and to the right may be seen a small group of parasites which have entered it from the lumen. They are clumped near its free border, are stained very deeply and appear to be of irregular shape and distinctly larger than the few parasites remaining in the lumen. From a nymph (230 C) the seventh day after engorgement (Table III).

Fig. 2. The section passes through the tips of epithelial cells extending out into the lumen in which intra-epithelial growth stages of the parasite in the zygote stage are visible noticeably larger than in Fig. 1. From a nymph (230 M) 12 days after engorgement (Table III).

Fig. 3. Still larger growth stages, which are of characteristically irregular outline, clumped together within the cytoplasm of an epithelial cell near the lumen of the intestine. From a nymph (242 B) 18 days after engorgement (Table III).

Fig. 4. Another cluster of growth stages observed in the same tick. It is to be noted that in this case their outlines are more evenly rounded.

Fig. 5. From another tick (240 H) taken the same eighteenth day after engorgement. The distal border of the epithelial cell, facing the lumen, is convex toward the left, while to the extreme right the nucleus of the cell appears as a large black mass. Just within the distal border are to be seen very dark oval structures which represent a stage in the life cycle of a protozoan which is distinct from the parasite of East Coast fever and is one of the complicating factors mentioned in the text. Between these protozoa on the left and the nucleus of the epithelial cell on the right are situated the large, irregular, intra-epithelial growth stages (zygotes) of the parasite of East Coast fever. Their diameter is almost twice that of the parasites represented in Fig. 4. They are less intensely stained and appear in some places to be joined together in a kind of syncytium.

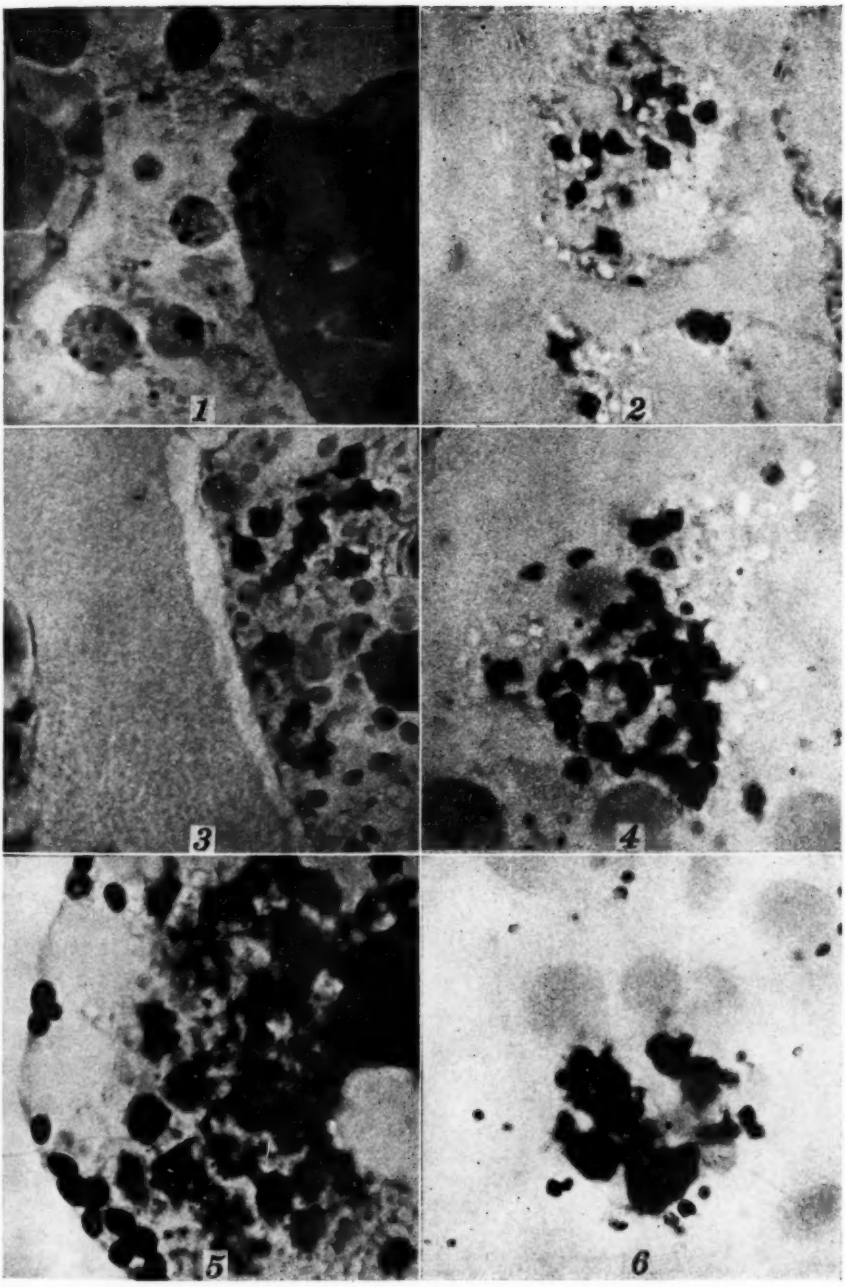
Fig. 6. In the centre of the figure the distal tip of an epithelial cell projecting into the lumen is cut across, the cell membrane of which is difficult to make out. It contains several zygotes which are transforming into motile oökinetes. The change is indicated by the appearance within them of distinct nuclei which in the photograph are jet black in a grey background. The left lower organism possesses two. From a tick which fed as a nymph (247 C) 23 days earlier, taken 5 days after it had moulted to form an adult female (Table IV).

PLATE II.

Fig. 7. A single free motile oökinete in an epithelial cell of the same tick is to be seen slightly to the left of the centre of the photograph. Its anterior extremity, containing a distinct black-looking nucleus, is pointing upward to the left, while its posterior end stretches out slightly downward and to the right. The nucleus of the parasite is approximately of the same size and shape as the nuclei represented in Fig. 6.

Figs. 8–14. All of these are from a smear of a tick (246 L 15) 28 days after engorgement and 4 days after moulting to an adult male (Table III). They illustrate some of the changes in the transformation of zygotes into motile oökinetes. In the centre of Fig. 8 may be distinguished a large somewhat rounded mass of material. Within its lower border and slightly to the left there is a nucleus which is not absolutely spherical but heart-shaped, as are the nuclei in the sections photographed in Figs. 6 and 7.

Fig. 9. Shows a parasite which is not so rounded, likewise in the centre of the photograph. It is easily recognisable by the presence of a typically heart-shaped nucleus in an area of the cytoplasm which is oval in shape and lighter than the remainder.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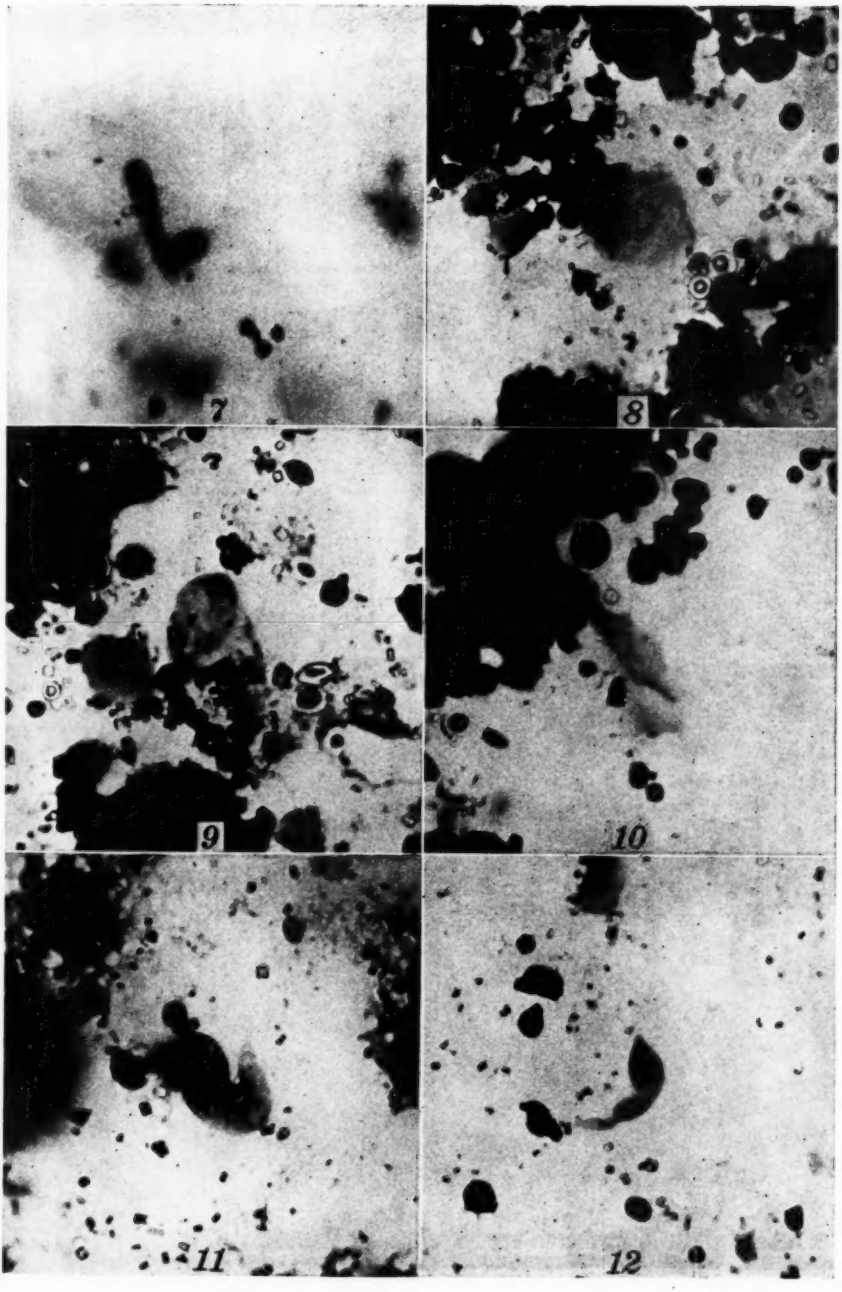
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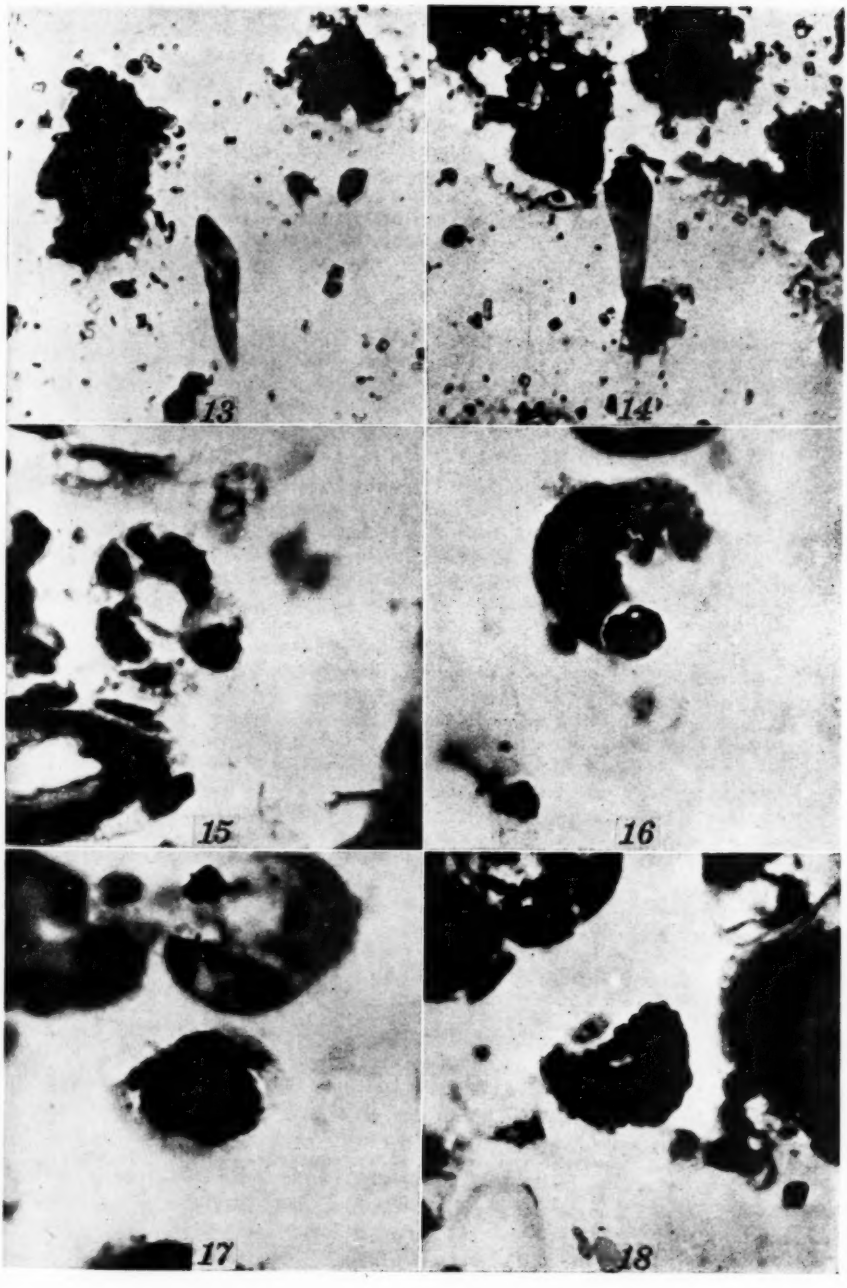
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Fig. 10. Illustrates a definite elongation of the parasite, identifiable again by its single nucleus which is of about the same size but more oval than heart-shaped. The parasite is in the centre of the figure and extends downward and to the right from a number of large black and grey masses. The irregularities in its outline are due to contact with other structures while the smear is drying.

Fig. 11. In the centre is a U-shaped parasite, which is now termed an oökinete since it is definitely longer than it is broad. The right limb of the U is the posterior extremity faintly outlined and extends upward to a sharp point. The left limb is bluntly rounded and much larger. It contains a distinct dark nucleus limited on the right by a small half-moon-shaped clear area.

Fig. 12. In the centre again is represented an oökinete whose head end is directed upward and whose tail passes downward and to the left. In the body of the parasite are two constrictions. In its head end is a darkly stained nucleus which is filamentous and almost wavy in shape.

PLATE III.

Fig. 13. This oökinete, the head of which points upward and contains a distinct nucleus, has attained its characteristic shape as a motile organism.

Fig. 14. Another typically motile form with head-end stretching upward. The nucleus is well defined. It is situated just within the cell membrane and to the right.

(Figs. 15–28 are all from sections.)

Fig. 15. Shows a portion of the salivary glands of a female adult (247 C) 5 days after moulting and 23 days after engorgement as a nymph (Table IV). To the left of the centre an acinus is visible and below and farther to the left a salivary duct. In the acinus the nuclei of the cells appear very black. A parasite (oökinete) may be distinguished within a salivary gland cell in the lower right-hand portion of the acinus. It is somewhat larger and more rounded than the nuclei and is characterised by the presence of a discrete spherical nucleus near its upper right-hand border. In the actual preparation its colour is also distinctive, the cytoplasm being stained blue and the nucleus crimson.

Fig. 16. Shows two salivary gland acini of an adult male tick (247 H) 7 days after moulting and 25 days after engorgement as a nymph (Table IV). Just above the centre is a salivary gland acinus and exactly in the centre a sharply defined black mass is to be seen. It is surrounded by a halo and contains several clear areas. This is a further stage in the intra-glandular development of a single parasite like that represented in Fig. 15.

Fig. 17. Salivary gland acini of an adult female tick (243 AA) 8 days after moulting and 32 days after engorgement as a nymph (Table II). Slightly to the left of the centre and a little below it is a portion of an acinus containing above an oval black-looking nucleus and below the nucleus and closely applied to it a large mass with beginning bud-like swellings about its periphery. This is an early sporont.

Fig. 18. In the centre is a sporont forming in a "clear" salivary gland cell. The outlines of the cell are not distinguishable, but a portion of its nucleus is seen slightly indenting the upper right margin of the sporont. The peripheral budding is further advanced than in Fig. 17. This is from an adult female (258 B) 10 days after moulting and 34 days after engorgement as a nymph (Table II).

PLATE IV.

Fig. 19. A further developed sporont in a "dark" salivary gland cell situated in the lower left part of the salivary acinus in the centre. The central part of the mulberry is beginning to clear, but the peripheral buds are deeply stained. From an adult male (250 P) 37 days after engorgement as a nymph, 12 days after moulting and 1 day after being placed on calf 5869 (Table III).

Fig. 20. Taken at the same magnification of a salivary gland cell so swollen up with parasites that it occupies more than double the area of the photograph. In the centre is a large sporont about the periphery and in the anterior of which the small infective sporozoites are being formed. From a partly engorged adult female naturally attached to an animal in an area where East Coast fever was common.

Fig. 21. Portion of another greatly enlarged salivary gland cell in which the sporont which is forming sporozoites is broken up into supplementary sporoblasts. Like Fig. 20, this is also from a naturally attached, partly engorged adult female.

Figs. 22 to 25 are all from the salivary glands of an adult female (250 AQ) 40 days after engorgement as a nymph, 16 days after moulting and 5 days after attachment to calf 5869 (Table III).

Fig. 22. Illustrating a large salivary gland cell almost completely filled with parasites. The nucleus of the cell is not visible. Just below it and to the left is the section of a duct. In the parasitised cell may be distinguished several fairly large sporoblasts developed from the sporont buds. Some of these masses can be seen to be forming small sporozoites about their periphery. Unattached sporozoites are very abundant.

PLATE V.

Fig. 23. Shows two salivary gland cells: a large one of irregular outline above to the right and a smaller roughly oval one below and to the left. In the upper cell there are more of the spherical sporoblasts giving rise to sporozoites and in the lower the sporozoites are more abundant and more densely packed together accompanied by only a few small sporoblasts.

Fig. 24. Two dense clumps of sporozoites in a single salivary gland cell. Same tick as Fig. 22.

Fig. 25. A salivary gland cell with its picnotic nucleus to the right and many sporozoites aggregated in a central mass and separated from the cell membrane by a clear area. A single sporoblast can be seen among the sporozoites above and to the left.

Figs. 26–28 are from the salivary glands of ticks which have ingested much blood.

Fig. 26. From a nymph (253 BM) 45 days after engorgement as a larva, 21 days after moulting and 12 days after attachment to calf 5840 (Table I). The central part of a large degenerate-looking acinus is illustrated. To the upper left is a cell discharging sporozoites downward to the right into the lumen of the acinus. The nucleus of the cell is an oval black mass near the left-hand margin of the photograph.

Fig. 27. A portion of an acinus with a cell containing many parasites. From a nymph (253 AJ) 39 days after engorgement as a larva, 15 days after moulting and 6 days after being placed on calf 5840 (Table I).

Fig. 28. A clump of apparently degenerate parasites being desquamated bodily into the lumen of an acinus. From a nymph (253 AX) 41 days after engorgement as a larva, 17 days after moulting and 8 days after attachment to calf 5840 (Table I). This concludes the cycle of those parasites in the tick which are not passed out in feeding.

PLATE VI.

The figures on Pls. VI and VII were drawn with a binocular Zeiss microscope 2 mm. objective 1·40 aperture, compensating oculars No. 20 and camera lucida giving a magnification at the level of the table of 3250 diameters.

Fig. 29, *a–g*. Red blood cells containing parasites in a blood smear dried in air, fixed in methyl alcohol and coloured by Giemsa's stain made from calf 5740 on which the ticks were fed that are listed in Tables II *et seq.* (p. 6).

Fig. 30, *a–x*. Other forms of the parasite which were seen in the same smear also within erythrocytes but which have been drawn without indicating the erythrocytes in order to save space.

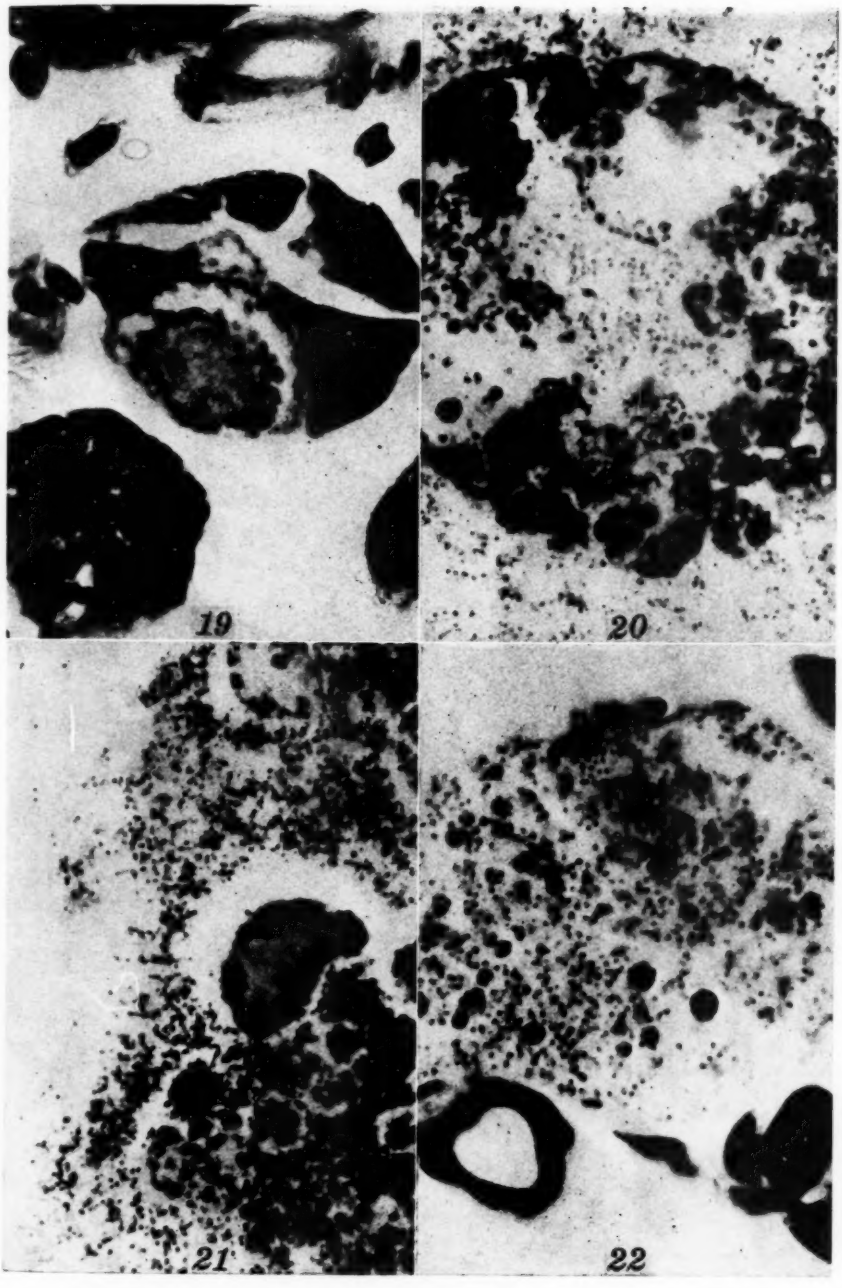
Fig. 31, *a–ab*. Parasites observed both within and without erythrocytes in a smear made from a nymph (223 D) the day that it dropped off engorged with blood. In order to avoid complications in Tables I–IV the daily smears are not listed. (First nymphal series continued in Table II.)

Fig. 32, *a–s*. Parasites in a smear of a nymph (225 B) 3 days after engorgement. (Second nymphal series, in Table III.)

Fig. 33, *a–q*. Parasites in a smear from a nymph (225 H) 5 days after engorgement. (Second nymphal series, Table III.)

Fig. 34, *a–x*. Parasites in a smear from nymph (230 F) 8 days after engorgement. (Second nymphal series, Table III.)

Fig. 35, *a–n*. Parasites in a smear from a nymph (228 Q) 12 days after engorgement. (First nymphal series, Table II.)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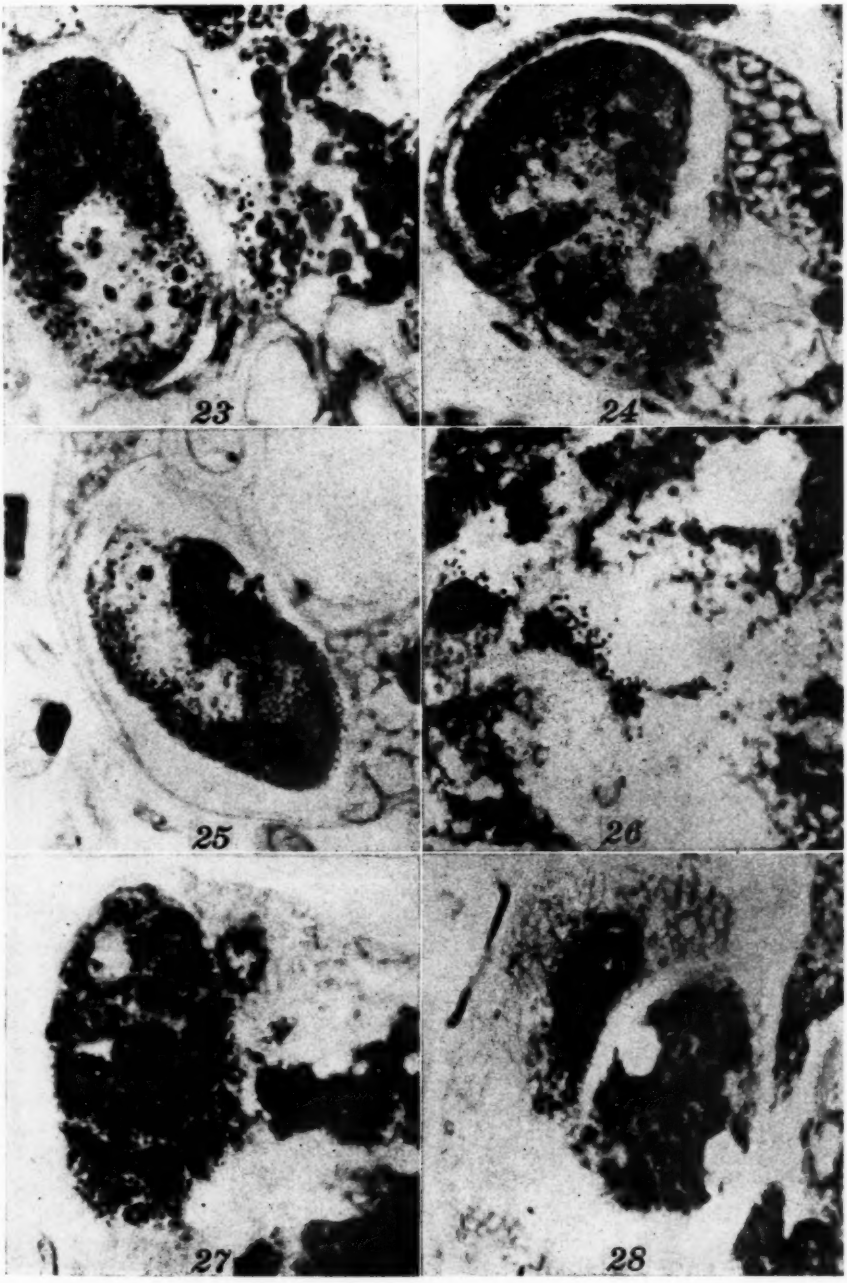

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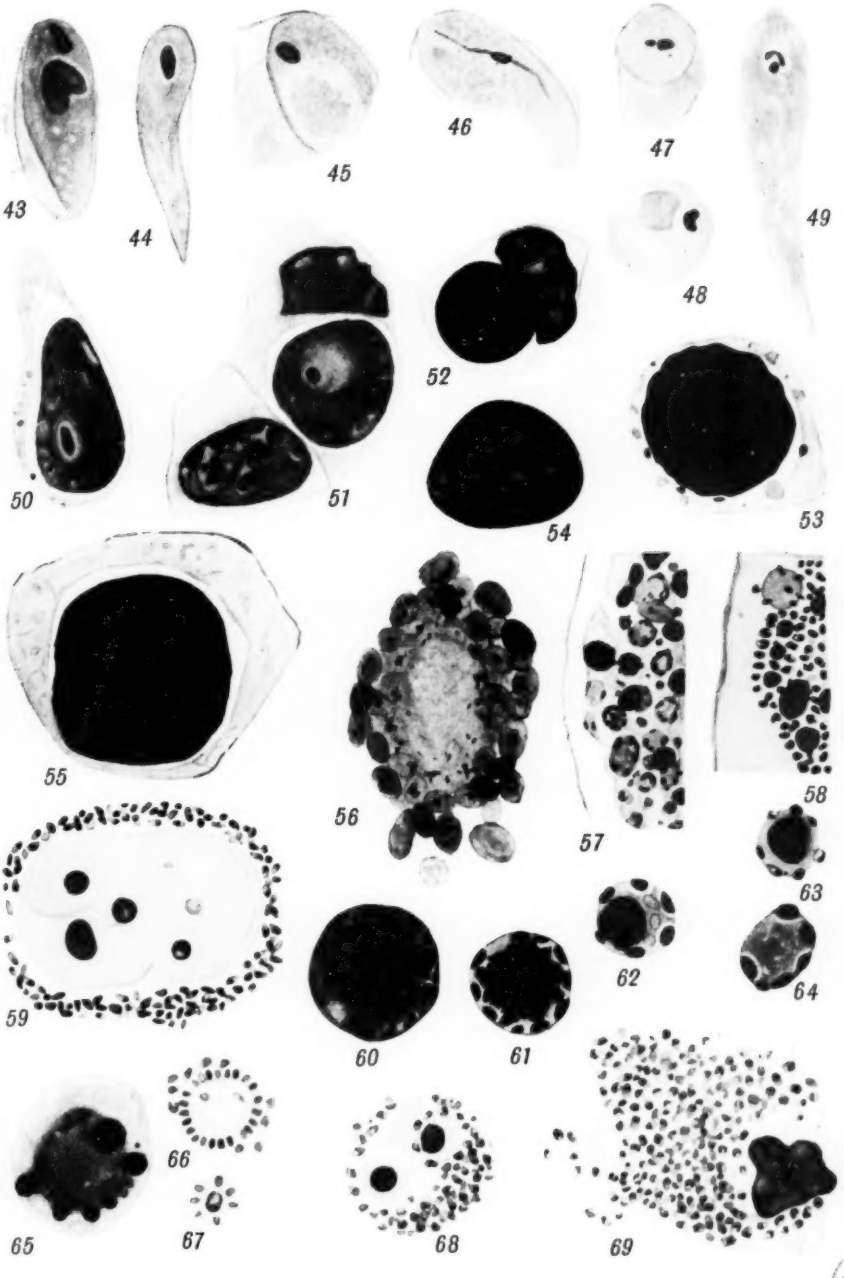
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(Figs. 36–69 are all from sections.)

Fig. 36, *a–n*. Parasites seen in the intestinal lumen of a nymph (232 D) 2 days after engorgement (Table IV).

Fig. 37, *a–b*. Parasites in pedunculated intestinal epithelial cells of a nymph (232 F) 3 days after engorgement (Table IV).

Fig. 38, *a–n*. Parasites in pedunculated epithelial cells of a nymph (230 C) 7 days after engorgement (Table III). *o–ac*. Parasites in the intestinal lumen of the same nymph. *ad–av*. Parasites in non-pedunculated epithelial cells of the same nymph.

Fig. 39, *a–f*. Parasites in non-pedunculated epithelial cells of a nymph (230 M) 12 days after engorgement (Table III).

Fig. 40, *a–j*. Zygotes in non-pedunculated epithelial cells of a nymph (230 BB) 18 days after engorgement (Table III).

Fig. 41, *a–k*. Zygotes in non-pedunculated epithelial cells of a nymph (242 B) 18 days after engorgement (Table III).

Fig. 42. Oökinete developing with a zygote from a tick (238 X) of the third nymphal series (Table IV) 19 days after engorgement and 1 day after moulting.

PLATE VII.

Figs. 43–48. From same ticks as Fig. 42.

Figs. 49–52. From a tick (247 C) of the third nymphal series (Table IV) 23 days after engorgement and 5 days after moulting.

Fig. 54. From a tick (243 M) of the first nymphal series (Table II) 25 days after engorgement and 1 day after moulting.

Fig. 55. From a tick (243 AA) of the first nymphal series (Table II) 32 days after engorgement, and 8 days after moulting.

Fig. 56. From a tick (258 B) of the first nymphal series (Table II) 34 days after engorgement and 10 days after moulting.

Figs. 57 and 58. From a tick (250 AA) of the second nymphal series (Table III) 38 days after engorgement, 14 days after moulting and 3 days after being placed on calf 5869.

Fig. 59. From a tick (258 Z) of the first nymphal series (Table II) 40 days after engorgement, 16 days after moulting and 4 days after being placed on calf 5199.

Figs. 60–65. From a tick (250 AQ) of the second nymphal series (Table III) 40 days after engorgement, 16 days after moulting and 5 days after being placed on calf 5869.

Figs. 66 and 67. From same tick as Fig. 59.

Figs. 68 and 69. From a tick (253 BG) of the larval series (Table I) 43 days after engorgement, 19 days after moulting and 11 days after being placed on calf 5840.

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LONGISTRIATA ŠKRJABINI N.SP. (NEMATODA, TRICHOSTRONGYLIDAE) FROM THE USSURI SQUIRREL.

By R. E. SCHULZ and M. P. LUBIMOV.

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(With 2 Figures in the Text.)

The 60th Union Helminthological Expedition (Skrjabin, Podjapolskaja and Schulz, 1929) to the Far East examined many squirrels (*Sciurus vulgaris mantshuricus* Thom. 1909), and among other helminths found a new species belonging to the genus *Longistriata* Schulz, 1926 (subfam. Heligmosominae Travassos, 1914). The same species had also been found by one of us (M. P. L.) in Ussuri squirrels at the Moscow Zoo.

Longistriata skrjabini n.sp.

Host: *Sciurus vulgaris mantshuricus* Thom.

Location: Small intestine.

Locality: Far East, U.S.S.R.

Description: *Longistriata.* The worms are coiled in a spiral in the anterior part of the body (2–3 turns). The head end is surrounded by a cuticular vesicula (Fig. 1). There are three weakly developed lips. Two pairs of papillae are seen on the head.

Behind the cephalic vesicula there are short ridges ranged as illustrated (see Fig. 1). The length of the ridges posterior to the vesicula is 0·015 mm. and 0·026–0·030 mm. in the middle of the body. The female has 28 ridges on each transversal circle, counting the adjacent row of ridges (in a zigzag)—56.

Male: 4·2–4·85 × 0·104–0·112 mm. (maximum breadth in the middle of the body). Cephalic vesicula slightly asymmetric, at times longer on one side than the other. Vesicula 0·041–0·060 × 0·037 mm. Head 0·022–0·023 mm. in diameter exclusive of the vesicula. Bursa (Fig. 2) 0·200 × 0·332–0·336 mm. Ventral rays pass in a common trunk and bend anteriorly; the latero-ventral reach the margin of the bursa, protruding it a little in the form of a sharp angle. The lateral rays arise from a common stem; the broad antero-lateral ray bends forward, the medio-lateral proceeds straight to the bursa margin and the postero-lateral, a thinner ray, is somewhat bent back. The externo-dorsal ray arises from a common trunk with the dorsal separating from the latter at a distance of 0·011 mm. from the basis and passing wave-like without reaching the bursa margin. The dorsal ray measures 0·074–0·090 mm. from the origin

of the externo-dorsal. Near its distal end (0·026 mm. from it) the dorsal ray is divided into two branches, each bifurcating, whereby the inner branches are longer than the outer. Spicules filiform, 0·415–0·422 mm. long, bifurcating near the proximal end into two branches of equal length, ending in a sharp point. Gubernaculum in the form of a groove 0·052–0·060 × 0·018 mm.

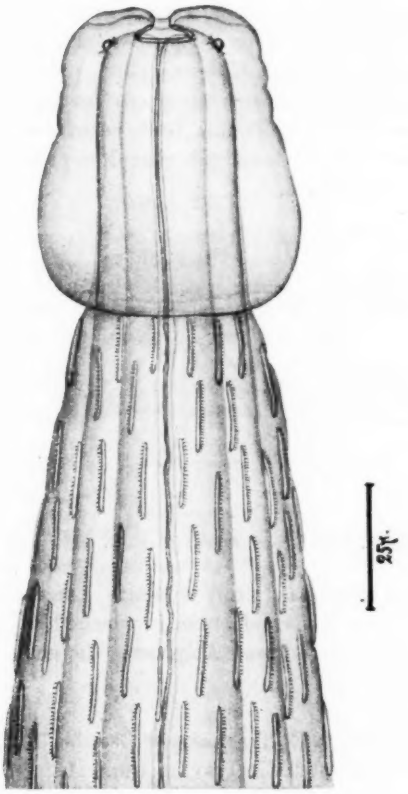

Fig. 1. *Longistriata skrjabini* n.sp., ♀, head end (scale = 25μ).

Female: 5·45–5·85 mm. long. Head 0·026–0·028 mm. in diameter excluding the vesicula, and 0·037 the vesicula included. Cephalic vesicula 0·060–0·061 mm. long. The oesophagus 0·400 mm. long. Vulva situated at a distance of 0·182–0·187 mm., in the form of a transverse slit 0·038 mm. long. Ovijector 0·187–0·232 mm. long. Diameter of body at vulva 0·093–0·097 mm., at anus 0·022–0·026 mm.

This nematode differs sharply from others of the genus by its characteristically short ridges, situated in chess-like order.

The former genus *Viannaia* was divided by Schulz (1926) into 3 subgenera:

(1) Sub-genus *Viannaia* (Travassos, 1914): cuticle transversely striated; longitudinal ridges or striations absent. Spicules relatively short. Gubernaculum absent or weakly chitinised. Parasites of marsupials and primates. Type-species: *Viannaia* (*Viannaia*) *viannaia* Travassos, 1914.

(2) Sub-genus *Trifurcata* Schulz, 1926: cuticle without marked transverse and longitudinal striation or ridges. Spicules massive, large (about $\frac{1}{5}$ of body length), trifurcating distally. Gubernaculum present, large. Parasites of Edentata. Type species: *Viannaia minuscula* Travassos, 1914.

(3) Sub-genus *Longistriata* Schulz, 1926: cuticle with longitudinal striation or ridges; spicules relatively long; gubernaculum present or absent. Parasites of Rodentia and Insectivora.

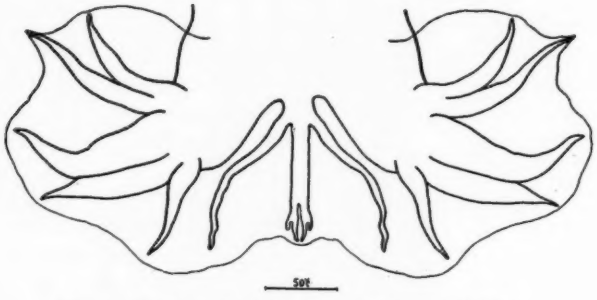

Fig. 2. *Longistriata skrjabini* n.sp., ♂, bursa (scale = 50μ).

Travassos and Darriba (1929) separate *Longistriata* from the genus *Viannaia* and consider it a valid genus, in which they place several species of the former genus *Heligmosomum*. At present the genus *Longistriata* includes 17 species as follows:

From Rodentia:

L. vexillata (Hall 1916) Travassos et Darriba 1929.
L. cristata (Gedoelst 1917) Trav. et Darr. 1929.
L. aculeata (Trav. 1918) Trav. et Darr. 1929.
L. nematodiriformis (Trav. 1918) Trav. et Darr. 1929.
L. alpha (Trav. 1918) Trav. et Darr. 1929.
L. beta (Trav. 1918) Trav. et Darr. 1929.
L. gamma (Trav. 1918) Trav. et Darr. 1929.
L. delta (Trav. 1918) Trav. et Darr. 1929.
L. wolgaensis Schulz 1926.
L. seurati Trav. et Darr. 1929.
L. leporis Schulz 1931.
L. nutria Gilbert 1931.
L. skrjabini Schulz and Lubimov 1931.

From Insectivora:

L. depressa (Dujardin 1845) Schulz 1926.

L. talpae (Morgan 1928) Schulz and Lubimov 1931.

From Marsupialia:

L. didelphis (Trav. 1914) Trav. et Darr. 1929.

From Primates:

L. dubia (Trav. 1921) Trav. et Darr. 1929 (= *Viannaia saimiris* Cameron 1923, fide Travassos et Darriba).

In our opinion not all of the foregoing species belong to the genus *Longistriata*. One of us (R. E. S.) hopes to discuss this question in a future paper.

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TWO NEW SPECIES OF TREMATODES FROM AN INDIAN RUFF (*PHILOMACHUS PUGNAX* GRAY).

By M. A. MOGHE, M.A., M.Sc.

From the Department of Zoology, College of Science, Nagpur, C.P., India.

(With 4 Figures in the Text.)

The two specimens of trematodes herein described were obtained from the intestine of an Indian Ruff, *Philomachus pugnax*. Of six birds examined, three harboured a few of both species. The genera to which these worms belong have not been hitherto recorded from India.

Echinostomum govindum n.sp. (Figs. 1–3).

Nine specimens were found in the rectal caeca; none was found elsewhere in the intestine.

The worms are elongate, nearly uniform in width, broadest on a level with the ventral sucker, taper in posteriorly. They measure 4·6 to 4·94 × 0·92 mm. (maximum width). The cuticle bears oblique spines directed outward and backward, more numerous on the anterior end, sparser over the region between the ventral sucker and the testes and absent on the post-testicular region.

The terminal anterior sucker (Fig. 1, *O.S.*), measuring 0·149 × 0·133 mm., is surrounded by a reniform collar (Fig. 2) of thirty-two hooks of uniform size (0·0254 mm. long) set at regular intervals except laterally where four hooks on each side tend to crowd together. Their arrangement in two rows is hardly recognisable.

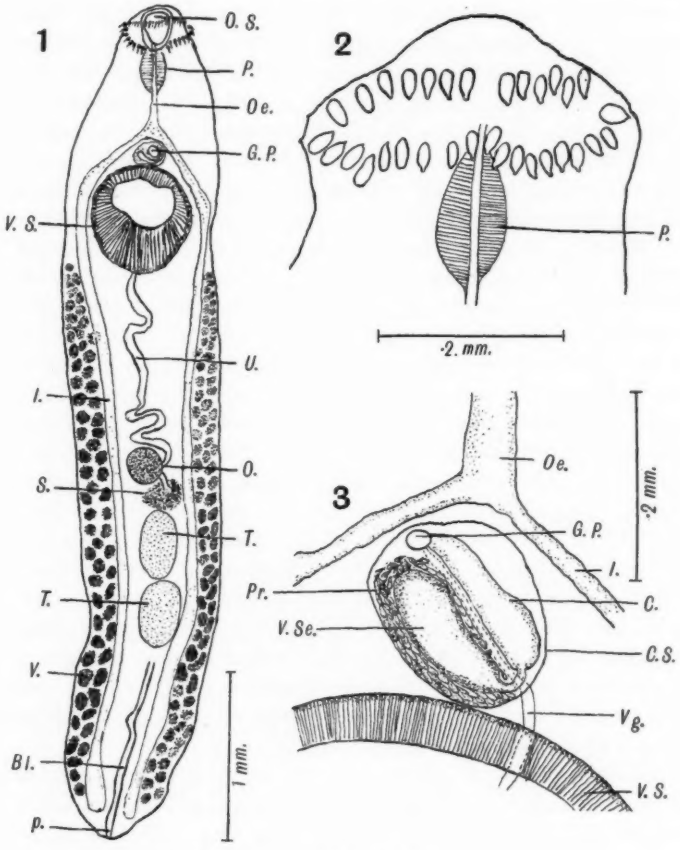
The mouth leads by a short pre-pharynx into a muscular pharynx (*P.*) measuring 0·22 × 0·36 mm., which is followed by a long oesophagus (*Oe.*), 0·26 mm. long, that bifurcates anteriorly to the ventral sucker into the intestinal caeca (*I.*). These encircle the ventral sucker (*V.S.*) and run backward to within 0·14 mm. of the posterior end of the worm.

The sub-globular ventral sucker lies about 0·96 mm. from the anterior end, well in front of the middle point of the body length. It measures 0·6 mm. in diameter, being larger than the oral sucker, the ratio of the two suckers being about 1 : 4.

The excretory pore (*p.*) lies at the hind end of the body and the system is Y-shaped. The posterior stem runs as far forward as the posterior testis where it branches.

The two testes (Fig. 1, *T.*) are ovoid bodies situated behind the ovary. Both are nearly of the same size (0·4 × 0·24 mm.), and lie one behind the other in

the median line. The testes and ovary lie in the posterior half of the body. The cirrus sac (Fig. 3, *C.S.*), an ovoid sac, lies below the intestinal bifurcation and the anterior border of the ventral sucker; it consists of a transversely elongated



Figs. 1–3. *Echinostomum govindum* n.sp.
Fig. 1. Ventral view.
Fig. 2. Anterior end to show arrangement of cephalic spines.
Fig. 3. Cirrus sac.

Key to lettering of figures.

Bl. Excretory bladder; *C.* Cirrus; *C.S.* Cirrus sac; *G.P.* Genital pore; *I.* Intestine; *O.* Ovary; *O.S.* Oral sucker; *Oe.* Oesophagus; *P.* Pharynx; *p.* Excretory pore; *Pr.* Prostate; *S.* Shell gland; *T.* Testes; *U.* Uterus; *V.* Vitellaria; *V.S.* Ventral sucker; *V.Se.* Vesicula seminalis; *Vg.* Vagina.

vesicula seminalis (*V.Se.*) surrounded by a prostatic gland (*Pr.*). The genital pore (Figs. 1 and 3, *G.P.*) is below the intestinal bifurcation, *i.e.* at the anterior extremity of the cirrus sac.

The ovary (Fig. 1, *O.*) lies 2·6 mm. from the anterior end of the body in the posterior half of the body length, anterior to the testes. It is rounded, much smaller (0·12 mm.) than the testes. In worms whose uterus contained no eggs, ovary was small, smaller than the shell gland. The latter (*S.*) lies between the ovary and the anterior testis, and in mature specimens fills the whole space between these two organs, being roughly of the same size as the ovary. A small oviduct arises from the ovary and enters the shell gland. The uterus (*U.*) arises laterally from the ovary and extends as a slightly convoluted tube up to the ventral sucker. The vagina (Fig. 3, *Vg.*) runs dorsal to the ventral sucker and the cirrus sac and opens in the genital pore. The vitellaria (*V.*) are rounded follicles and extend from the posterior end of the intestinal caeca to the anterior level of the ventral sucker. Eggs (in balsam) measure 0·047 × 0·023 mm.

This parasite, having a reniform collar beset with spines, belongs to the Echinostomidae Looss 1902 and to the genus *Echinostomum*, because the hooks on the head crown are uninterrupted, the suckers are fairly close together, the cirrus sac lies entirely in front of the acetabulum, the testes lie in the posterior half of the body and the vitelline glands do not extend beyond the posterior border of the ventral sucker. Nearly 120 species of this genus have so far been recorded. I have been able to get the descriptions of most of the species, *e.g.* of those recorded by Johnston (1928), Barker (1915), Nicoll (1913 and 1914), Odhner (1911), Looss (1899), Dietz (1910), Lutz (1924), and Kotlán (1922). Besides, the table of characters of the different species of this genus given by Skrjabin (1915) and the descriptions of the species described by him (1915) were also available to me. In no species so far recorded do the cephalic spines number 32 except in *E. australe* Johnston (1928) from the Australian water hen, *Gallinula tenebrosa*. The species described in this paper differs from Johnston's in length and width of the body, size of suckers and hooks, and size of eggs. Besides, the ovary in Johnston's species lies in the anterior half of the body. I consider it, therefore, a new species which I name *Echinostomum govindum* with the following specific diagnosis:

Length 4·6 to 4·49 mm.; maximum width at the level of the ventral sucker 0·92 mm.; oral sucker 0·133 ×0·149 mm.; surrounded by a reniform collar of 32 hooks, each 0·0254 mm. in length; ventral sucker 0·6 mm. in diameter; ratio of the two suckers 1 : 4; testes and ovary in the posterior half of the body; testes (0·4 × 0·24 mm.) in a line one behind the other, posterior to the ovary; ovary 0·12 mm. in diameter; eggs (in balsam) 0·047 × 0·023 mm.

Host: *Philomachus pugnax* Gray.

Location: Rectal caeca of host.

Locality: Nagpur, C.P., India.

Paramonostomum microstomum n.sp. (Fig. 4).

Ten specimens of this parasite were collected from the posterior part of the small intestine. Its body is elongated, the anterior part incurved ventrally. The anterior end is obtuse and the posterior bluntly rounded. The worms are

1·348 mm.–1·624 mm. long. The posterior two-thirds of the body is fairly uniform in width. Maximum width 0·375–0·409 mm. anterior to the testes.

The small oral sucker (Fig. 4, *O.S.*) is nearly terminal and has a diameter of 0·063 mm. The ventral sucker is absent. There is a very short oesophagus and no pharynx. The intestine bifurcates at an acute angle and diverges posteriorly enclosing the ovary, the uterine coils and the cirrus sac. The intestinal caeca extend to within 0·05 mm. of the posterior end of the worm.

The testes (Fig. 4, *T.*) lie in the posterior region outside the intestines, one on each side of the body, *i.e.* they are extra caecal; they are elongate and lobed, 0·13–0·15 mm. long. The cirrus sac (*C.S.*) lies in the anterior half of the body between the intestinal caeca, it is 0·413 mm. long. Posteriorly it contains a straight and narrow vesicula seminalis and terminates in the genital pore (*G.P.*) which lies immediately below the intestinal bifurcation very near the anterior end. The ovary (*O.*) lies in the median line between the testes, inside the in-

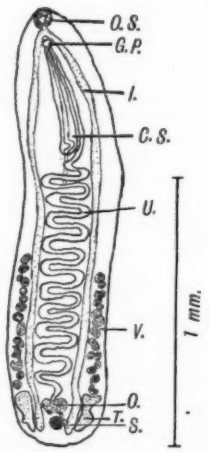


Fig. 4. *Paramonostomum microstomum* n.sp. Ventral view.
(See key to lettering under Figs. 1–3.)

testinal caeca; it is an elliptical structure from which a small oviduct arises to join the shell gland situated posterior and slightly lateral to it. The uterus (*U.*) runs anteriorly from the ovary and forms 13 to 15 loops on either side. The vagina runs alongside the cirrus sac being nearly as long as the latter, it opens in the genital pore. The uterus extends along more than half (0·9 mm.) the length of the body. The vitellaria extend from the middle of the uterine region to about the anterior end of the testes. The eggs have long polar filaments and measure (in balsam), without their terminal filaments, 0·051 mm. in length.

Referring to the key given by Harrah (1922, p. 13), this monostome belongs to the family Notocotylidae which according to him comprises two sub-families (p. 49): Notocotylinae and Nudocotylinae. The distinction between the two is

based on the position of the genital pore. The species described in this paper belongs to the family Notocotylidae which comprises three genera, two of them, viz. *Notocotylus* and *Catatropis*, possess protrusible or non-protrusible ventral glands. The third genus *Paramonostomum* is characterised by the absence of these glands and to this genus must belong the worm here described. This genus was created by Lühe (1910) to hold *Monostomum alveatum* (Mehlis) Ceplin; subsequently Harrah (1922, p. 55) described other species, *P. echinum* and these are the only two species of this genus so far recorded. The following table gives the important differences between the three species:

	P. alveatum	*P. echinum*	*P. microstomum* n.sp.
Length	0·6 to 1·0 mm.	2 to 2·5 mm.	1·348 to 1·625 mm.
Max. width	0·4 to 0·7 mm.	0·6 to 0·7 mm.	0·375 to 0·409 mm.
Body spines	—	On anterior half, 5 μ in length	Absent
Oesophagus	—	Short	Very short
Diameter of oral sucker	0·05 to 0·08 mm.	102 to 105 μ	0·063 mm.
Cirrus sac	—	Extends from genital pore to the beginning of the second third of the body	Slightly smaller in length than *P. echinum*
Vagina	—	Half as long as the cirrus sac	Nearly as long as the cirrus sac
Eggs	19 to 21 μ by 8 to 10 μ	20 μ in length, length twice as width	0·051 mm. in length, length two and half times the width
Host	*Anas penelope, Anser anser, Cygnus cygnus*, etc.	*Fiber zibethicus*	*Philomachus pugnax*
Distribution	North Germany, Europe	America	India

The above table shows that the monostome from *Philomachus pugnax* differs from the others and I conclude it is a new species which I name *P. microstomum*, with the following specific characterisation:

Length 1·348 to 1·625 mm.; maximum width 0·375 to 0·409 mm.; diameter of oral sucker 0·063 mm.; oesophagus very short; body devoid of spines; vagina as long as the cirrus sac; extent of the uterus more than half the body length; eggs 0·051 × 0·02 mm.

Host: *Philomachus pugnax* Gray.

Location: Small intestine of the host.

Locality: Nagpur, C.P., India.

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PRECIPITIN AND SKIN TESTS AS AIDS IN DIAGNOSING TRICHINOSIS.

By DONALD L. AUGUSTINE and HANS THEILER.

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(With Plate VIII, containing Figs. 1, 2, and Fig. I in the Text[1].)

Introduction.

Prior to 1928 a number of attempts were made to perfect a serological method for the diagnosis of trichinosis. None, however, was successful, and the inconclusive results and failures were due apparently to the use of saline, alcoholic and ether extracts of heavily infected muscle as antigens.

In 1928, Bachman reported a number of experiments which uniformly demonstrated antibodies in laboratory animals infected with *Trichinella spiralis* or immunised with a saline extract of powdered trichinella larvae previously isolated from all host proteins. Precipitins were also detected in one infected human subject who presented typical clinical symptoms of trichinosis. Later Bachman (1928*a*) demonstrated a clearly defined, delayed type of skin reaction in laboratory animals after less than a week's infection with *Trichinella spiralis*. It appeared that this test might prove effective for diagnosing the infection in its early stages, whereas the precipitin test was found of value only in established infections.

Bachman's results with laboratory animals were promising indeed and, in view of the fact that there are neither practical nor reliable means of detecting trichinosis in swine as a sanitary measure, and that the absolute diagnosis of the disease in man is often difficult or impossible to establish, the present study was undertaken to determine the value of the precipitin test and skin tests, described by Bachman, as exact and practical methods for diagnosing this infection in these hosts.

The writers wish to express their gratitude for the courtesies and co-operation received during the present investigation from the Department of Public Health of Massachusetts through Dr Clarence L. Scamman and the several City Health Directors of Massachusetts, particularly from Dr Ernest M. Morris, Director of the Fall River Department of Health, and Mr Melvin W. Brown, Superintendent of the Town Farm, Wakefield, Massachusetts. Through the kind invitation of Mr N. C. Marshall, Resident Manager of the South American

[1] Figures occurring on plates bear Arabic numbers, whilst text-figures bear Roman numbers.

Gold and Platinum Company, Andagoya, Colombia, the senior author was able to investigate certain phases of the problem under tropical conditions. We are indebted to Mr Marshall and the members of the Company for their whole-hearted co-operation, and are especially grateful for the constant assistance and helpful advice received from Dr W. H. White, the resident physician, with whom the work done there was directly associated. Cordial acknowledgment is made for aid received in Panama from Dr Louis Schapiro, Field Director of the Rockefeller Foundation, and from Dr J. C. Mattatall, Supervising Veterinarian of the Canal Zone.

METHODS.

The antigen used in the present study was prepared according to the method described by Bachman (1928). The trichinella larvae were obtained from guinea-pigs which were previously infected by being fed trichinous meat. About 30 days after infection, when the larvae had located in the muscles, the pigs were killed. The skin and viscera were removed and the carcass was washed and passed through a food-grinder with a fine knife. The ground meat was then digested in a solution of 0·4 per cent. pepsin and 0·3 per cent. hydrochloric acid. The solution was frequently stirred and kept in an incubator at 38° C. for about 15 hours, after which time the meat was almost completely digested and the trichinella larvae were free and alive. The solution was then passed first through a sixteen-per-inch mesh and finally through a forty-per-inch mesh which separated the larvae from the fine particles of undigested meat and bits of bone. The larvae were allowed to sediment and the filtrate containing fine particles of digested meat was removed with a siphon. Repeated washings with tap water and finally with distilled water practically separated the larvae from all host protein. The larvae at last were collected in sedimentation jars and transferred to a small agate mortar and placed in an incubator at 38° C. until they had formed dry flakes. These flakes of dried larvae were placed in ether for 24 hours, then in a vacuum over sulphuric acid for another 24 hours, after which the flakes were pulverised and the powder was either immediately extracted with Coca's solution (NaCl 0·7 per cent., $NaHCO_3$ 0·05 per cent. and phenol 0·4 per cent. in distilled water) or sealed in sterile glass ampules, each containing either 150 or 250 mg., and kept in an electric refrigerator for future use.

The suspension of trichinella powder in Coca's solution was kept 3 days in an electric refrigerator, after which time the liquid was separated from the sediment by passing it through a Seitz filter No. 3. The filtrate was then transferred to sterile vaccine bottles and closed with rubber stoppers of special forms and perforated almost to the top whereby a hypodermic needle could be readily inserted.

REACTIONS IN LABORATORY ANIMALS.

Preliminary to our observations on man and swine a number of intradermal and precipitin tests were made on guinea-pigs and rabbits. Trichinosis was established in these hosts by forcibly feeding them trichinous meat. Subsequent

to these feedings the skin and precipitin tests were carried out at various intervals. Bachman (1928) observed that some of his normal animals gave positive skin reactions after several such tests had been made on them. We also noted this reaction in several of our uninfected rabbits, and in one instance after three skin tests had been made. To avoid this occurrence in the infected groups, both rabbits and guinea-pigs, only one skin test was made on each animal of a series, and no skin tests were made prior to infection.

The skin tests were made on the abdomen of guinea-pigs, after the hair had been removed with a depilatory powder and the site washed with alcohol. This test was also made at first on the abdomen or sides of the rabbits, but later on the backs of the ears which were more convenient to work with and proved equally as sensitive. For testing these animals, 0·1 c.c. of a 1 per cent. dilution of the trichinella antigen was injected intracutaneously in the prepared site. For the control test on guinea-pigs an equal amount of the extracting fluid, Coca's solution, was injected about 5 cm. distant from the antigen site. With rabbits this control test was made on the ear opposite that injected with antigen. The antigen was tested at the beginning of the experiment on guinea-pigs and rabbits with established trichinosis and produced well-defined and typical positive reactions.

The course of positive skin reactions has been similar in both rabbits and guinea-pigs. A thickening of the skin at the site of the injection of the antigen was noted after the first half-hour, which gradually increased in thickness and diameter until, about 1 hour later, it usually had reached its maximum, involving, in some instances, an area from 3 to 4 cm. in diameter. The oedema is at first solid with distinct margins, often up to 5 mm. in thickness, usually more or less hyperaemic and haemorrhagic immediately surrounding the needle puncture. Twenty-four hours after injection the oedemic area may be slightly enlarged but the oedema by this time is generally flaccid and usually without an erythema. The oedema disappears slowly, and 48 hours after injection it is usually entirely reduced and only a small reddish blue area remains at the site of injection.

Nine guinea-pigs and fourteen rabbits were used in this preliminary experiment, and the skin tests subsequent to the feeding of trichinous meat were made on the second, fifth, seventh, ninth, fifteenth, twenty-eighth, thirty-first and thirty-third days respectively. The earliest positive skin reaction observed among the guinea-pigs occurred on the ninth day after infection. The remaining five pigs were examined on the fifteenth day after infection and all developed positive reactions. Of the two rabbits examined on the fifth day, one developed a typical positive reaction, while the second showed but a slight thickening of the skin at the site of the injection of antigen. Three rabbits gave indefinite reactions on the seventh day. On the ninth day three other rabbits gave typical positive reactions. The remaining rabbits of the series were examined at later dates and gave well-defined, positive tests with the exception of two which developed weak, doubtful reactions.

On the fifteenth day of infection and at varying intervals thereafter, samples of blood were taken from the heart of the guinea-pigs and rabbits to test their sera for precipitins. The antigen was diluted from 1 : 100 to 1 : 3000 with Coca's solution and about 0·3 c.c. undiluted serum in precipitin tubes was overlaid with an equal amount of the different dilutions of antigen. The tubes were left at room temperature and read an hour later. In many instances, however, a precipitate had already formed and was plainly visible within 20 or 30 min. Control tests were made with Coca's solution and normal rabbit or guinea-pig serum, antigen and Coca's solution, and antigen and serum of rabbit or guinea-pigs with established trichinosis and known to contain precipitins.

The first positive precipitin reaction in guinea-pig serum occurred on the thirty-third day after infection. A faint, though clearly visible, precipitate was noted in an antigen dilution of 1 : 1000 and was clearly defined in the antigen dilution of 1 : 100. Only one of the nine guinea-pigs showed precipitins in their sera at this time, although all were noticeably sick. Three died on the following day and showed numerous trichinella larvae in their tongues and diaphragms. The remaining six guinea-pigs were again tested on the forty-third day of infection. At this time all gave well-defined reactions in an antigen dilution of 1 : 1000, the highest dilution used. These guinea-pigs were then killed and used in the preparation of antigen. All showed heavy infections with *Trichinella spiralis*.

Precipitins were detected somewhat earlier in three of the infected rabbits. Two of these gave on the twenty-eighth day of infection faint rings in an antigen dilution of 1 : 1000 and definite, clear-cut rings in an antigen dilution of 1 : 100. The third rabbit gave a faint ring in a dilution of the antigen of 1 : 100. Three others gave definite positive reactions on the thirty-fifth day with an antigen dilution of 1 : 1000. The last examination was made on the forty-third day after infection and the remaining eight rabbits all gave clear-cut, positive reactions in an antigen dilution of 1 : 1000. These rabbits were then killed to make antigen. All showed heavy infections.

The results here obtained agree with those of Bachman (1928) with the exception that our skin tests on infected animals usually did not give a definite positive reaction until after the ninth day of infection, although a typical positive reaction was observed as early as the fifth day in one infected rabbit. We believe the difference may be due primarily to Bachman's rabbits having been tested once or twice prior to infection, thereby producing a local hypersensitivity, a condition observed by Bachman in some of his control rabbits, and by us in a normal rabbit after three skin tests had been made. In our series of skin tests on infected guinea-pigs and rabbits, only one test was made on each individual.

Attempts were made to demonstrate the skin reaction by the scratch method, *i.e.* by placing a few drops of the test antigen (1 : 100 dilution) on the skin and then making a few superficial scratches. These, as well as ophthalmic tests, made with the same dilution of antigen, were negative in both infected and normal rabbits and guinea-pigs.

Reactions in man.

The course of a typical positive skin reaction observed in man is decidedly different from that observed in laboratory animals, and is of the immediate type (Fig. 1). Within 3–5 min. after injecting the antigen a blanched, elevated wheal with a seemingly flat surface appears; the wheal more or less rapidly increases in area until after 1 hour it has usually reached its maximum, at which time it varies from 10 to 20 mm. in diameter in different individuals. The wheal is generally more or less regular in outline, but may in some instances produce narrow, pseudopodial outrunners equal in length to the diameter of the wheal. An erythema surrounding the wheal usually develops shortly after the formation of the wheal and is often quite pronounced within 5 min. after injection of the antigen. An areola, 6 cm. broad, more or less solid in colour, fading in intensity at the periphery or sometimes decidedly mottled, has been observed in some instances at the height of the reaction. There has been practically no oedema observed at this period, and the wheal stands out quite blanched against the reddish background. After the first hour the wheal invariably grows less definite and gradually becomes indistinguishable from the band of erythema. Twenty-four hours later the site of the reaction is usually marked by a more or less circumscribed area, slightly pink, and varying from 5 to 8 cm. in diameter. Some induration at times has been noted, but the oedema has only been slight, if any. No swelling of the regional glands nor any interference with the use of the arm has been noted.

Through the co-operation of the Massachusetts State Health Department and Boston Hospitals the authors were notified of suspected or reported cases of trichinosis in man. On the first nine cases which came to our attention the skin tests were made with the antigen in a dilution of 1 : 100. Of these, seven presented typical clinical symptoms of trichinosis and gave definite histories of having eaten pork about 1 week before the onset of symptoms. One case was seen by us in the third week of illness, or approximately 30 days after eating trichinous pork. Three others were seen between 3 and 4 months after infection and the remaining three cases were not seen until 6 months after infection. These seven gave positive skin reactions and their sera were positive for precipitins in an antigen dilution of 1 : 1000. Precipitin tests were not made with higher dilution of the antigen in this group. Controls for both the skin and precipitin tests were negative. Faecal examinations were made only on one individual. No evidence of intestinal parasites was found.

The two remaining cases of this group were hospital cases suspected of having trichinosis by the attending physicians. Aside from a complaint of muscular pains their clinical symptoms and histories were not at all characteristic of trichinosis. Both gave negative precipitin tests for trichinosis and one gave a negative skin reaction. The second showed a very slight reddening at the site of injection of the antigen which disappeared entirely within 2 hours. Stool examinations on these two cases were negative for intestinal parasites.

These results, together with the character of the clinical symptoms and personal histories, do not favour the diagnosis of trichinosis. The slight skin reaction in the one case was either suggestive of a past light infection from which the skin still retained its sensitivity or a normal hypersensitiveness to the trichinella protein.

As a control on the tests made on the reported cases of trichinosis, examinations were made on five adult individuals who, as far as they were aware, never were infected with trichinella. The same dilution of antigen (1 : 100) was used in the skin tests on these persons. Three of this group developed positive skin reactions, showing early wheals with erythema. They further showed marked induration with oedema 72 hours after injection of the antigen, and the skin was hot and itchy. The control tests with Coca's solution were negative. The precipitin tests were all negative.

There are two possible explanations for the lack of correlation between skin and precipitin reactions in the three so-called normal persons: (*a*) natural hypersensitiveness to the trichinella antigen, or (*b*) the result of previous unrecognised trichinosis in which the serum had lost its detectable antibodies although the skin still retained its sensitivity. In view of the possibility that anyone without restricted diet in the United States might have eaten undercooked trichinous pork at one time or another, the question of trichinosis in our so-called normal persons could not be ruled out. It therefore appeared that this point could be definitely determined only by running the tests on persons known to be trichinella free.

It has long been known that trichinosis is exceedingly rare or absent among the native population of the tropics, and for this reason it seemed advisable to carry on the tests under such an environment. In addition, it was hoped to test the antigen against infections with other parasites, this not being feasible in New England and best carried out in a country rich in parasitic fauna but lacking trichinella.

Through an invitation of the South American Gold and Platinum Company such an environment was made available, and one of us (D. L. A.), with the co-operation of Dr W. H. White, the resident physician, conducted the tests at the Company's hospital at Andagoya, Colombia, situated about 150 miles up the San Juan River at its confluence with the Condoto River. Aside from the cleared area occupied by the company itself and small neighbouring villages, the country is a veritable jungle, with its native population settled entirely along the banks of the rivers. The natives are Choco Indians and descendants of the early Spaniards who sought gold there, and their slaves. Much of the unskilled labour of the company is done by native negro help, while some of the more technical work is done by negroes from Panama, Jamaica and other West Indian islands. Supervision of the work is in charge of white Americans and Europeans.

That trichinosis does not exist in the native population of this part of the world is shown by the autopsies of Dr Herbert C. Clark of the Gorgas Memorial,

Panama. In the more than 4000 autopsies done at the Gorgas Hospital and 500 in other parts of Central America, mostly in Honduras, Dr Clark found but three infections with trichinella, and these occurred in Italians. In two of these the cysts were calcified, indicating an infection of long standing (personal communication). It is therefore evident from Dr Clark's observations that trichinosis, at least among the native human population, is exceedingly rare, if not non-existent, in these parts of the Americas. Neither was trichinella observed by us in pigs of Panama and Colombia, nor in seven rats killed at the abattoirs of Istmina and Condoto, small native villages about 5 miles distant from Andagoya.

Most of the tests at the camp hospital were made on native patients, including employees or members of their families and residents of the district, a few Indians, and men, apparently in good health, who had presented themselves for a physical examination before being accepted in the employment of the company.

The physical examinations were all made by Dr William H. White, the resident physician of the camp. Blood smears were made on each individual for malaria and relapsing fever, and a specimen of the faeces was examined for intestinal parasites. The blood smears and faecal specimens were prepared for examination by trained laboratory assistants and were examined by either Dr White or the senior author.

The first forty Colombian cases were skin tested with the antigen in a dilution of 1 : 100, the same as used in the earlier Boston work. The results of these tests, together with the data from the stool and blood examinations and the precipitin tests, are included in Table I. This table shows that twenty-eight individuals gave positive skin reactions. A decided variation was noted in the intensity of these positive reactions. Many wheals were but slightly larger than that caused by the control solution and without an erythema, while others were similar to or severer than reactions observed in known cases of trichinosis.

The positive skin reactions were not, however, in accord with the precipitin tests. As will be noted in Table I, only seven of the twenty-eight with positive skin reactions were positive by the precipitin test. It will further be noted that three of the nine individuals with negative skin reactions gave positive precipitin tests. While it was not possible to rule out trichinosis entirely from this community, the complete lack of correlation between the skin and precipitin tests indicated that the positive reactions might be due to some non-specific factor. It was suggested that the presence of other parasites might be responsible, but since the incidence of parasites noted was practically identical in both positive and negative groups (Table I), this as a factor was apparently of no pertinent importance.

The marked variation in the intensity of the skin reactions of this group suggested the possibility of a natural hypersensitiveness of some individuals to the trichinella protein. For instance, in five persons the reactions caused by the trichinella protein were barely differentiated from the reaction caused by

the injection of Coca's solution, while in four others the antigen produced wheals of 2–3·4 cm. in diameter with pseudopodial outrunners and surrounded by erythema from 2 to 4 cm. broad. The remaining nineteen positive skin tests were of an intermediate character.

To test this, the next group of Colombian cases, consisting of seventy-five individuals, were skin tested with varying dilutions of the antigen, namely 1 : 250, 1 : 500, 1 : 1000, 1 : 1500, 1 : 2000, 1 : 5000 and 1 : 10,000. Precipitin tests, and blood and faecal examinations for the presence of parasites were also made, as in the previous group. Usually two skin tests with different dilutions of the antigen were made on each individual.

The results of these skin tests with the antigen in dilution up to and including 1 : 5000 were quite similar to those obtained earlier with the antigen in a dilution of 1 : 100, and showed no correlation between the skin and precipitin reactions; also that the incidence of parasites in all groups was not pertinently significant.

Table I. *Analysis of forty Colombian skin tests with a* 1 : 100 *dilution of antigen.*

		Precipitin tests		Blood examinations		Stool examinations				
						Positive				
	No.	Positive	Negative	Positive	Negative	Hookworm	Ascaris	Trichuris	Amoebae	Negative
Positive skin tests	28	7	21	1 (malaria)	27	18	10	13	4 (*E. coli*)	5
Negative skin tests	12	3	9	2 (malaria)	10	12	8	4	—	1

It was noted, however, that fewer positive reactions developed with the higher dilutions of antigen and that these were markedly milder in character. The wheals were smaller and often without surrounding erythema. For example, of nine persons skin tested with the antigen in 1 : 250 and 1 : 5000 dilutions, six developed positive reactions with the more concentrated solution, while only two developed positive reactions with the higher dilution, and these, as is shown in Fig. I, were noticeably less severe.

Twelve persons were skin tested with antigen dilutions of 1 : 250 and 1 : 10,000. Eight gave positive reactions to the dilution of 1 : 250. One of these, a girl who apparently was particularly sensitive to the trichinella protein, developed a wheal 20 × 12 mm. from which extended a pseudopodial outrunner 8 mm. in length. The wheal was surrounded by an erythema 15 mm. wide. This girl also developed a slight reaction to the 1 : 10,000 dilution of the antigen. The wheal developing from the injection of this solution was in reality slightly less in diameter than the "blister" produced from the control solution, but the erythema surrounding it was about twice as broad (0·5 mm.) as the

erythema surrounding the lesion provoked by the injection of Coca's solution. The reaction faded within the first hour and became indistinguishable from the control. It was recorded as doubtful. All others of this group were negative to the 1 : 10,000 dilution of the antigen.

Because the actual number of observations with the antigen in a solution of 1 : 10,000 on supposedly trichinella-free individuals is small, it cannot be concluded that it will consistently exclude all normally hypersensitive persons. However, the one so-called doubtful reaction noted with this dilution was so strikingly slight that its identity as a pseudo-reaction would not easily be mistaken.

While the antigen in a dilution of 1 : 10,000 appeared promising in excluding persons normally hypersensitive to the trichinella protein, its effectiveness in detecting trichinosis remained unknown.

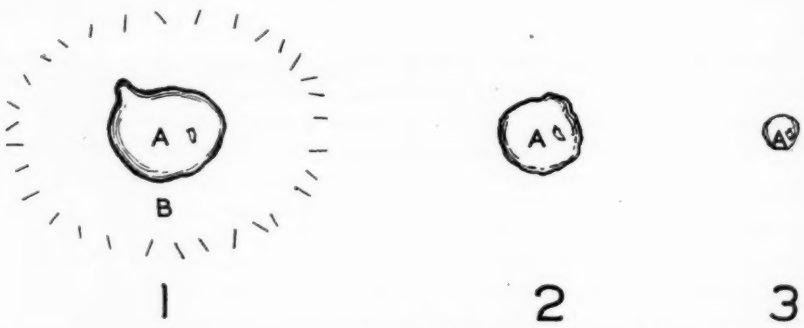

Fig. I. Outline drawing of skin reactions to trichinella protein induced by dilutions of 1 : 250 and 1 : 5000. Both tests were made simultaneously on one individual: (1) 1 : 250; (2) 1 : 5000; (3) control, Coca's solution. A, wheal; B, erythema.

To determine this, skin tests were made after our return to Boston on hospitalised cases of trichinosis and on recent cases reported to the State Department of Health using high dilutions of the test antigen. The results of these tests are summarised in Table II. This table shows that all except one, No. 101, gave positive skin reactions, with a 1 : 10,000 dilution of the antigen. The reactions with this dilution of the antigen ran a similar course and were as definite in character as those obtained with the test antigen in a dilution of 1 : 100, but noticeably milder, and when seen on the following day were scarcely distinguishable. Figs. 1 and 2 are water-colour paintings of the reaction observed in Nos. 107 and 108, Table II, and represent the condition at 5–7 min. after injecting the antigen. The failure to develop a typical skin response in case No. 101, Table II, is probably due to the short duration of her infection. Exact information as to the time of infection was, however, lacking, and permission to repeat the tests at a later date was not obtained.

Three skin tests were made with higher dilutions of antigen, two, Nos. 110 and 111, Table II, with the antigen in a dilution of 1 : 15,000, and one, No. 109, Table II, with a dilution of 1 : 50,000. This table shows that the dilution of 1 : 15,000 produced a typical reaction in No. 110 but failed completely in No. 111. Permission to repeat the test with a 1 : 10,000 dilution of antigen, and to obtain blood for a precipitin test in No. 111, was not granted. Case No. 109, Table II, gave a typical reaction with the antigen in a dilution of 1 : 10,000, but did not respond to the injection of the antigen in a dilution of 1 : 50,000. From these observations it therefore appears that the trichinella

Table II. *Skin tests in clinically diagnosed trichinosis performed with high dilutions of antigen. Massachusetts cases.*

No.	Approximate duration of infection	Skin test. Dilution of antigen 1 : 10,000	1 : 15,000	1 : 50,000	Precipitin test	Remarks
101	14 days	–	Not made	Not made	–	Skin test showed very slight erythema surrounding wheal which was no larger than control. Re-examination at later date not possible
102	16 „	+	„	„	+ –(1 : 100)	Son of 104. Precipitin ring not definite
103	18 „	+	„	„	+(1 : 100)	Son of 104. Thin, but definite precipitin ring
104	3 weeks	+	„	„	–	Father of Nos. 102 and 103
105	3 „	+	„	„	Not made	Brother of No. 106
106	3 „	+	„	„	+(1 : 1000)*	Sister of No. 105
107	4 „	+	„	„	Not made	See Fig. 1
108	5 „	+	„	„	+(1 : 2000)	See Fig. 2
109	10 „	+	„	–	+(1 : 2000)	Biopsy positive
110	12 „	+	+	Not made	+(1 : 2000)	Biopsy eight weeks after infection. Intense infiltration subcutaneous tissue and corium, plasma cells. Fibrosis, no larvae. Hospital case. Brother of No. 111
111	12 „	Not made	–	„	Not made	Sister of No. 110. Very light infection, and did not require medical attention

* Highest dilution run.

antigen in a dilution of 1 : 10,000 is an effective and promising means of detecting trichinosis (in man), but in higher dilutions it appears to have but little practical application, if any.

As previously noted in Table I no correlation could be established between the precipitin tests and the skin tests in the first forty persons examined in Colombia. Neither was there any relationship detected between the precipitin tests and the presence of parasites noted since the incidence of the parasitic species encountered was practically identical in both the positive and negative precipitin groups. Throughout the Colombian work these results were constant, and of the 115 complete examinations there (including skin and precipitin

tests and blood and faecal examinations), twenty-seven gave positive precipitin reactions. It early appeared that the positive precipitin reactions might be due to some non-specific factor or factors, since the rings formed at the interphase of the serum and antigen were, in many instances, flocculent in character and up to 3 mm. wide, whereas the rings noted in sera from trichinous individuals were sharp, not over 1 mm. wide and never flocculent. Furthermore, nine of these Colombian cases showed precipitins in the control tubes when the sera were overlaid with Coca's solution, but not when overlaid with normal saline.

The first intimation that one of these factors might be either a chemical or a drug taken internally previous to the withdrawal of blood for the precipitin test came when the serum of a girl poisoned with bichloride of mercury gave heavy, flocculent precipitates with dilutions of the antigen up to 1 : 3000 (higher dilutions not run) and in the control tube of Coca's solution (Table III). Again, two European employees with malaria, one with benign tertian and the other with aestivo-autumnal, likewise showed heavy precipitates in their sera with the same dilutions of the antigen and in the control tube with Coca's solution. The rings formed within 15 min. and those in the control tubes were

Table III. *Analysis of Colombian positive precipitin tests.*

	With history of recent medication			Without definite history of recent medication
	Quinine	Novarsenobillon	Bichloride of mercury	
No.	16	6	1	4*

* Case 1. Severe pinta of 5 years' duration. Case 2. Chronic appendicitis. Case 3. Femoral abscess. Case 4. No history obtained, not a patient.

the first to appear. An examination of their hospital cards showed that each had received 10 grains of quinine at dredge camps before coming to the hospital, and 15 grains of quinine intramuscularly, 10 grains of quinine and 4 of plasmoquin compound tablets per os at the hospital prior to our taking the blood samples. An examination of the hospital record cards of patients previously tested for precipitins showed that those with positive reactions had received recent medication, usually 24 hours prior to our tests, either with quinine, plasmoquin compound or novarsenobillon.

To determine if the presence of these drugs in the blood may have caused the precipitation in the tests noted, it was planned to run precipitin tests before and after the patients received such medication. Unfortunately, during our stay at Andagoya, no malarial cases came to the hospital without previously having taken quinine. Malaria seldom originated at the main camp at Andagoya, and employees at the dredges received quinine in case of fever from the dredge masters before being sent to the main hospital for observation at Andagoya. Among the native population, it is the custom to take patent medicines upon the least provocation of illness, and as malaria is one of the common diseases of this part of the country, most of the patent medicines are

reputed to contain quinine. Therefore, it was not possible to exclude quininisation before hospital treatment, although the patient gave no history of having taken quinine before his entrance to the hospital. However, through the kindness of Dr W. H. White, we were able to gain definite information on this point after our return to Boston. An employee of the company who had heretofore always resided in Idaho, contracted aestivo-autumnal malaria, exactly 35 days after his arrival at the camp. Dr White took a sample of blood before medication and a second sample after the patient had received 70 grains of quinine per os, at the rate of 30 grains per day. The sera from these blood samples were sent to us by air mail and were received in perfect condition. The serum taken before quininisation was negative while that taken after was positive in a 1 : 1000 dilution of the antigen. The control was negative.

Similar results were obtained at the Camp Hospital with serum from a patient with plavas or tertiary yaws. Samples of blood were obtained before and after intravenous injection with novarsenobillon. The serum from the blood taken before administration of novarsenobillon was negative, while that obtained on the morning following injection of the drug showed precipitates with the antigen up to and in a dilution of 1 : 1000 (the highest dilutions of antigen tested) and in the control, Coca's solution. Here the precipitate in the control tube formed first and was the most prominent.

From these observations it appears that the administration of certain drugs may cause precipitation with the trichinella antigen. Attempts to demonstrate this phenomenon in laboratory animals, by injecting rabbits intravenously and intramuscularly with quinine dihydrochloride, were not successful, because of the marked susceptibility of rabbits to this drug. Further observations and experimentation are required on this point.

Reactions in swine.

Table IV gives the results of a series of skin and precipitin tests made on eight pigs, five of which previously had been fed bits of trichinous rabbit meat. These pigs were purchased from a town farm piggery about 15 miles north of Boston, and were kept at the piggery isolated from other pigs throughout the experiment. They were fed upon garbage collected from a nearby town.

Precipitin tests were run on each pig before the trichinous meat was given. All of these tests were negative, and control tests run on rabbit sera known to contain precipitins were positive. Subsequent precipitin tests were made at frequent intervals, the blood being withdrawn from either the tail or heart prior to injecting the antigen for the skin test. No skin test was made before infection. The skin tests performed at various intervals after infection were made on the inner surface of the right hind-leg. The control test, Coca's solution, was made approximately 3 cm. distant from the site of the injected antigen. In this series 0·1 c.c. of the 1 : 100 dilution of antigen was injected.

The course of typical positive skin reactions was found strikingly different

from that observed in man and in rabbits and guinea-pigs (Fig. 2). The reaction, however, resembles that occurring in man in that it is of the immediate type. Within 5 min. after injecting the antigen a fine reddish line appears around the periphery of the wheal which is at first no larger than that caused by the injection of a similar amount of Coca's solution, the control, but which gradually increases in area until after about 30 min. it may be from two to three times its original diameter. Within 10 min. after injection the wheal is usually already dark purple with clear-cut margins and without any appreciable

Table IV. *Intradermal and precipitin tests on swine experimentally infected with* Trichinella spiralis.

0·1 c.c. of a 1 : 100 dilution of the test antigen was used for these intradermal tests.

Pig No.	Days after infection: 6 S*	6 P*	11 S	11 P	20 S	20 P	35 S	35 P	56 S	56 P	91 S	91 P	Parasites found at autopsy
529†	–	–	Not made	Not made	Not made	–	+	+	.	.	.	.	*T. spiralis* (one larva per field in diaphragm). *Balantidium, Trichomonas, Entamoebae*
539†	Not made	–	+– (slight)	–	„	–	+	+	.	.	.	.	*T. spiralis* (eight larvae per field in diaphragm). *Balantidium, Trichomonas, Entamoebae*
536‡	„	–	Not made	–	+	–	+	+	.	.	.	.	*T. spiralis* (twelve larvae per field in diaphragm). *Oesophagostomum dentatum*
518§	„	–	„	–	Not made	–	+	+	+	+	.	.	*T. spiralis* (two larvae per field in diaphragm). *Balantidium* and *Trichomonas*
534‖	„	–	„	–	„	–	Not made	+	Not made	+	+	+	*T. spiralis* (one larva per field in diaphragm). *Oesophagostomum dentatum*
Three controls	.	.	.	.	.	.	.	.	.	.	–	–	*Oesophagostomum dentatum*—stool examinations only

* *S*=skin test; *P*=precipitin test.
† Killed on thirty-sixth day.
‡ Died on thirty-seventh day.
§ Killed on fifty-sixth day.
‖ Killed on ninety-second day.

erythema or oedema of the tissues immediately surrounding it. Considerable fading may be observed as early as an hour after injection and, usually, after 24 hours, practically no trace of the reaction remains. The reaction in pigs is therefore of the immediate type whereas that observed in guinea-pigs and rabbits more nearly approaches the delayed type.

No progressive changes have been observed in our control tests on pigs with Coca's solution. The blister first formed gradually disappears, usually within 20 min. after injection.

As shown in Table IV the earliest skin reaction occurred on the eleventh day following ingestion of the trichinous meat, pig No. 539. The wheal produced by the antigen was, however, but slightly larger than that brought about by Coca's solution. Further, the coloration was light, and although considered a positive reaction at that time, it has since been considered doubtful, in view

of the course of what have been considered typical positive reactions observed later in the experiment. Pig 536 gave a definitely positive reaction on the twentieth day after infection. Although skin tests were not made on the other pigs at this time, it is believed that in general typical skin reactions develop at this time, and probably may develop as early as the fifteenth day after ingestion of trichinous flesh. Pig 534 was not skin tested until the ninety-first day after infection. The reaction observed at this time was as definite as those noted for the twentieth and thirty-fifth days after infection on other pigs of the series.

At this time (35 days after infection) a skin test was made on pig 534 with an *Ascaris* antigen prepared from whole pig Ascarids by the same method used in the preparation of our trichinella antigen. For the test 0·1 c.c. of a 1 : 100 dilution of this antigen was injected intradermally. Five minutes later the site showed marked inflammation with oedema. Twenty minutes after injection the inflammatory oedema involved an area about 6 cm. in diameter and was of a deep purple colour. The deep coloration persisted for 2 hours, after which time observations were discontinued until the following day, when the inflammation and oedema had disappeared and the site was marked by a faded purple blotch. A similar reaction was also observed on young pigs taken from a nearby pen. An examination of the faeces of these pigs by the salt flotation method showed the presence of *Oesophagostomum* sp. alone. Faecal examination of other pigs at the farm revealed no *Ascaris* infections, and it was concluded that the severe reaction to the ascaris protein was not due to any sensitivity derived from an ascaris infection.

Precipitin tests were made on all the infected pigs at various intervals. As shown in Table IV all gave strong positive reactions on the thirty-fifth day in an antigen dilution of 1 : 1000. In the last precipitin test, made on the ninety-first day of infection, pig 534 was likewise positive in an antigen dilution of 1 : 2000. Three young, apparently normal, pigs from a neighbouring pen were tested at this time and were negative by this test.

The five infected pigs were either killed or died from natural causes on days noted in Table IV. At autopsy all showed trichinella infections of various degrees of intensity determined by microscopic examinations of slips of muscle from the pillars of the diaphragm. An examination of the organs was made and samples of faeces were examined by smear and salt flotation method to determine the presence of any parasites. The results of these examinations are included in Table IV.

Following this study on experimentally infected pigs, tests were made on a number of pigs at a farm near Fall River. This piggery was selected because three cases of trichinosis in man were known to have originated from it. Two large hogs which were to be slaughtered within the next few days and seven younger pigs chosen at random as they fed upon swill were tested intradermally, using 0·1 c.c. of a 1 : 100 dilution of the trichinella antigen. One of the smaller pigs developed a typical positive reaction while the others, including the two

large hogs, were negative. Blood was taken from the pig showing a positive reaction and the serum collected was positive for precipitins in an antigen dilution of 1 : 1000. Tests with higher dilutions of the antigen were not made.

In order to confirm these positive reactions, the pig was purchased, killed, and a search made for the encysted trichinella in the muscles. Over eighty slips from the tongue, diaphragm, intercostals and masseters were examined microscopically, but no larvae were found. These muscles were then ground and digested in a solution of 0·4 per cent. pepsin and 0·3 per cent. hydrochloric acid, as done in the preparation of the antigen. Fifteen hours later the undigested material was removed by screening and the filtrate allowed to sediment. In the sediment 507 living trichinella larvae were obtained. The results of this survey indicate that the skin and precipitin tests are delicate and far more accurate than muscle examination. Other parasites found at autopsy in this pig were *Oesophagostomum dentatum*, *Balantidium coli*, *Entamoeba* sp. and *Trichomonas* sp.

Twenty-one pigs were tested in Colombia, South America, including thirteen from the corral of the South American Gold and Platinum Company at Andagoya (Choco), four at the abattoir at Istmina and four at the abattoir at Condoto, towns within a radius of 6 miles of Andagoya. The skin tests were made with a 1 : 100 dilution of the trichinella antigen. Three of the thirteen pigs examined at Andagoya gave typical positive skin reactions, but all were negative by the precipitin test. The three pigs with positive skin tests were killed and their muscles were first examined microscopically and later by the digestion method previously described. No trichinella larvae were found. Two other pigs of the same lot, both with negative reactions, were examined when slaughtered in a similar manner and were likewise found negative for trichinella. Tongues of the eight pigs tested at the village abattoirs were examined by the same methods, but no trichinella larvae were found.

Stephanurus dentatus and *Oesophagostomum dentatum* were found in all five pigs killed at Andagoya. Faecal specimens were obtained from four of the remaining eight pigs of the same lot and these were positive for *Oe. dentatum*. No other parasites were found. The eight pigs at Istmina and Condoto were killed for the market the day after our tests were made and no faecal specimens were obtained from them for examination.

The three positive skin reactions observed among the Andagoya pigs indicated a normal hypersensitiveness since trichinosis could be ruled out, for, as far as is known, trichinella is non-existent there. It was determined therefore to further test the antigen in higher dilutions to which normally hypersensitive animals might fail to react and yet be effective in producing a positive reaction in trichinosis infections.

An opportunity was offered to test this point at the abattoir at Panama City, where twenty-three pigs from the interior of Panama were examined. Four intracutaneous injections in 0·1 c.c. amounts of the following dilutions of antigen were used on each pig, 1 : 100, 1 : 1500, 1 : 5000, and 1 : 10,000. As

shown in Table V, four pigs gave positive reactions in an antigen dilution of 1 : 100 but were negative with the higher dilutions. Two other pigs gave positive reactions in as high as a 1 : 5000 dilution of the antigen. The reactions with this dilution were markedly less pronounced than in the 1 : 1500, and those produced by the latter were less pronounced than those caused by the 1 : 100 dilution of antigen. None reacted with a dilution of 1 : 10,000, the highest dilution tested. Precipitin tests were made on the six pigs with positive skin reactions and on five others which failed to react to this test. These precipitin tests were all negative. Microscopic examination of muscles from these eleven pigs, whose data are included in Table V, failed to show any evidence of trichinosis.

Table V. *Skin tests with high antigen dilutions and precipitin tests on Panama pigs.*

Data on twelve additional pigs with negative skin tests not included.

Pig. No.	Dilution of antigen 1 : 100	1 : 1500	1 : 5000	1 : 10,000	Control (Coca's)	Precipitin test	Parasites found
554	+	–	–	–	–	–	*Oesophagostomum dentatum*, Coccidia
555	–	–	–	–	–	–	*Ascaris lumbricoides, Stephanurus dentatus.* Unidentified nematode egg
556	–	–	–	–	–	–	*Ascaris lumbricoides, Stephanurus dentatus, Oesophagostomum dentatum*
557	–	–	–	–	–	–	*Ascaris lumbricoides, Oesophagostomum dentatum.* Unidentified nematode egg as in No. 555
558	+	+	+	–	–	–	*Oesophagostomum dentatum, Stephanurus dentatus*
559	–	–	–	–	–	–	*Oesophagostomum dentatum*, Coccidia
560	–	–	–	–	–	–	*Ascaris lumbricoides, Stephanurus dentatus*
563	+	+	+	–	–	–	*Ascaris lumbricoides, Stephanurus dentatus.* Unidentified nematode egg as in Nos. 555 and 557
564	+	–	–	–	–	–	*Oesophagostomum dentatum*, Coccidia
567	+	–	–	–	–	–	*Ascaris lumbricoides, Stephanurus dentatus*
573	+	–	–	–	–	–	*Oesophagostomum dentatum*, Coccidia

Intradermal reactions with an antigen dilution of 1 : 10,000 were further tested on pigs at a town farm near Boston. Nine young pigs, averaging about 60 lb. each, of the regular stock were first bled from the heart to obtain sera for precipitin tests and were then injected intradermally with 0·1 c.c. of a 1 : 10,000 dilution of trichinella antigen. All skin tests were negative. The precipitin tests made on the following day were also negative, while a control test, run with serum from a rabbit with established trichinosis, was positive in a 1 : 2000 dilution of the antigen. The pillars of the diaphragms were obtained when the pigs were slaughtered for market, 3 days after the skin tests were made. These were first examined microscopically and later by digestion and sedimentation.

No trichinella larvae were found. Stool examinations were not made. From these observations, therefore, it appears that pigs normally hypersensitive to concentrated solutions of trichinella antigen do not react when the test is made with the antigen of a 1 : 10,000 dilution. Whether this is generally true is not known, it can be determined only after many observations have been made.

Skin tests with high dilutions of the test antigen were made on four pigs fed varying amounts of trichinous meat. The data relative to this experiment are recorded in Table VI. Pigs 511 and 545 were kept at the town farm throughout the experiment, while Nos. 508, 540 and 541 were purchased from the town farm while they were still sucklings and kept thereafter at the Animal House, Harvard Medical School. Before feeding them trichinous meat, precipitin tests for trichinosis were made on the serum of each pig, and all appeared negative by this test. Pigs 511 and 545 were each fed an entire hind leg of a trichinous rabbit. Pig 508 was fed half a flank of a trichinous guinea-pig and No. 541 was fed a quarter of the flank of the same guinea-pig. Subsequent data are included in Table VI.

Table VI. *Skin tests with high antigen dilutions and precipitin tests on trichinous pigs.*

Pig No.	Days after infection	Skin tests 1 : 100	1 : 10,000	1 : 15,000	Precipitin test (highest dilution)	Remarks
511	10	+ –	+ –	Not made	Not made	Killed, muscles heavily parasitised. Thirty-five larvae per field in diaphragm
	32	+	+	,,	,,	
	83	+	+	,,	+ (1 : 2000)	
545	10	+ –	+ –	,,	,,	Killed, muscles heavily parasitised. Thirty-five larvae per field in diaphragm
	32	+	+	,,	,,	
	83	+	+	,,	+ (1 : 2000)	
508	20	+	+	+ –	Not made	Killed. Three larvae per field in diaphragm
	35	Not made	Not made	Not made	+ (1 : 2000)	
541	20	+	+	+ –	Not made	Kept alive for experimental purposes
540	Control	–	–	–	–	Muscles negative

As shown in Table VI, the antigen in a dilution of 1 : 10,000 was equally effective as a dilution of 1 : 100 in producing a typical positive reaction in the infected hogs. The reaction produced by the 1 : 10,000 dilution of antigen appeared as early as that caused by the 1 : 100 dilution but it was less pronounced and involved a slightly smaller area (Figs. 1 and 2), and disappeared completely 4 hours after injection. Twenty-four hours after injection a faint salmon-reddish spot about 3 mm. in diameter was noted at the site of injection. The reaction due to the 1 : 100 dilution of antigen followed the usual course.

It will also be noted in Table VI that pigs Nos. 508 and 541 were skin tested with the antigen in a dilution of 1 : 15,000; the reaction from this dilution was, however, of a doubtful nature. The coloration, although definite, was lighter than that produced by the antigen in a dilution of 1 : 10,000 and the area involved at the height of reaction was but slightly greater than that produced by

the control, Coca's solution. This dilution was therefore considered too high for practical purposes and no further tests with it were made.

Pigs Nos. 511 and 545 were killed on the eighty-third day of infection and showed an average of thirty-five trichinella per microscopic field (low power) in the diaphragm muscles. Pig No. 508 was killed on the thirty-fifth day of infection and showed a relatively light infection, averaging three larvae per microscopic field in the muscles of the diaphragm. Pig No. 541 was kept alive for experimental purposes.

From these observations it is apparent that the skin test made with a 1 : 10,000 dilution of the antigen is effective in diagnosing trichinosis in swine, even in light infections, and it also appears specific since, as far as our observations have gone, non-trichinous pigs, hypersensitive to more concentrated solutions of the antigen, do not respond to it.

DISCUSSION.

In the work under consideration skin tests and precipitin reactions were studied in nine guinea-pigs, fourteen rabbits, seventy swine and 140 human subjects. To arrive at an evaluation of the tests for diagnostic purposes we sought to perform them under various conditions upon trichinous and non-trichinous individuals of all of the species of animals used. As was expected the amount of antigen in the test dose would play a part in the skin tests, and hence a number of dilutions of the testing fluid were employed. The effect of the presence or absence of unrelated intestinal parasites had to be taken into account, and for this purpose it became necessary to perform the tests on individuals in a heavily parasitised community in which, however, *Trichinella spiralis* was not likely to be encountered. For this reason some of the investigations were carried out in Colombia and in Panama, where parasitic infections are common, but *Trichinella spiralis* extremely rare. For the purpose of discussion, the work may be divided into a consideration of the reactions obtained in experimental and natural trichinosis, in normal individuals, and in individuals with other parasitic infections. Further, questions concerning the specificity, the delicacy and the applicability of the tests, and an attempted interpretation of non-specific and anomalous reactions will be taken up.

The results obtained in experimental infections in guinea-pigs and rabbits agree closely with those obtained by Bachman (1928) and do not call for extended discussion. Like Bachman we observed that normal laboratory animals may become sensitised when subjected to a number of skin tests, and for this reason and because previously untested normal animals never reacted to a first test, our animals were not skin tested before infection. In one instance an infected rabbit gave a clear-cut positive reaction on the fifth day, two other infected rabbits gave indefinite reactions on the seventh day, but usually rabbits and guinea-pigs regularly reacted after the ninth day of infection. The control tests with the fluid used for antigen extraction and with the antigen on normal

animals were invariably negative. The first positive demonstration of specific precipitins in the sera of guinea-pigs and rabbits occurred on the thirty-third and twenty-eighth day respectively after the animals were infected. In the experimentally infected hogs the earliest positive skin reaction was recorded on the eleventh day in the first group of animals (see Table IV). In the later group (see Table VI) two of the animals gave indefinite (+ −) reactions on the tenth day after infection, but gave clear-cut positives on the thirty-second day. Two other swine of the same group gave typical reactions on the twentieth day. It was not possible to demonstrate the presence of precipitins in the sera of these experimentally infected hogs on the twentieth day, but on the thirty-fifth day after infection all those tested gave clear-cut reactions at dilutions up to 1 : 1000. In no case, in any of our precipitin tests, was the end-point of the reaction determined.

The natural infections studied include twenty human infections. On the basis of rather vague and unconvincing histories and the presence of muscular pain, trichinosis was suspected in two cases by the physicians in charge. We were not able to demonstrate the presence of specific precipitins in either of these cases; one gave no skin reaction, while the other showed but a transitory reddening of the site of injection. Were these to be accepted as cases of trichinosis the histories obtained would make it necessary to assume that the infections were of considerable duration, must have been extremely light, and the symptoms necessitating medical attention at the time of our tests in no way related to the earlier unsuspected and undiagnosed trichinous infection. Experience has shown that established cases of trichinosis of more than 4 weeks' duration do not fail to give a skin reaction to a suitable test dose of the antigen, and that precipitins can be shown to be present in the sera of such individuals. It thus seems improbable that these two cases are to be regarded as trichinous infections. The transitory reddening obtained may have been due to slight hypersensitivity of obscure origin. In the remaining eighteen cases, the history of the ingestion of pork, the clinical symptoms and in some cases the positive biopsy finding, leave no doubt as to the correctness of the diagnosis, and in these cases positive skin tests were obtained in all the individuals tested except two. The two exceptions were Nos. 101 and 111 (see Table II). In the former the test was performed 14 days after the ingestion of the infected pork with an antigen dilution of 1 : 10,000; the precipitin test made at the same time was also negative. Unfortunately it was not possible to retest this individual at a later date. In No. 111 the infection was so mild that the woman did not require the services of a physician; a precipitin test was not made and no skin reaction was obtained with an antigen dilution of 1 : 15,000. The shortest interval of time after which a positive skin reaction was obtained in an infected human subject was 16 days after infection (No. 102, Table II); the precipitin test was doubtful. Besides No. 101, the only other failure to demonstrate the presence of specific precipitins was in No. 104, which was a case of 3 weeks' standing. In view of the results obtained in the experimental infections in

animals, it is not surprising that it was not possible to obtain a positive precipitin test on the serum of this individual. The earliest positive demonstration of precipitins in a human subject occurred 18 days after infection; a positive reading was given only at the highest concentration (1 : 100) used. In one case (No. 104) a failure was recorded at 3 weeks.

The only natural case of trichinosis encountered in animals other than man was in a hog. Pork obtained from an animal in this herd was known to have given rise to three human cases of trichinosis. It was intended to make a survey of this herd of swine, but the conditions under which the animals were kept made this impractical at the time and hence only the animals to be slaughtered and a number of young pigs selected at random were tested. Of the nine animals tested, one gave positive results in both the skin and precipitin tests. Artificial peptic digestion of a sample of muscle was the only method by which it was possible to verify trichinous infection in this animal, which could not be shown to be infected by the usual microscopic technique. Negative skin and precipitin tests were obtained on nine hogs in a local piggery, and the absence of larvae of *Trichinella spiralis* in the musculature was confirmed by digestion of the diaphragms of these animals.

Non-specific skin reactions were obtained in three out of twenty-one pigs tested in Colombia and in eleven out of twenty-three tested in Panama, but none of the sera from these animals yielded positive results to the precipitin test. There was no significant difference in the parasitic fauna of the animals that gave skin reactions and those that did not. Similarly non-specific skin reactions were obtained in twenty-seven out of the first forty human subjects tested in Colombia and proportionately the same number of reactions were obtained in a later group of seventy-five individuals tested. As in the case of the hogs, there was no marked difference between the parasitic fauna of the reactors and that of the non-reactors. In addition, non-specific reactions followed injection of the antigen in three out of five normal persons tested in Boston. In the Boston cases the possibility of light, unsuspected trichinous infections could not be ruled out, but it is reasonably certain that the individuals on whom observations were made in Colombia had at no time been infected. The reactions in the Boston cases, obtained by means of the more concentrated antigen (1 : 100), though atypical, may thus be an indication of such a previous infection, even though the presence of precipitins in the blood serum of these individuals could not be established. On the other hand a non-specific sensitisation may be the explanation of the phenomenon in these cases, as it undoubtedly is in the Colombian reactors. That the sensitisation in the latter is non-specific in character is borne out by the fact that in eight out of twelve persons tested with antigen dilutions of 1 : 250 and 1 : 10,000 only one gave a reaction at the higher dilution. This reaction was, however, not typical and was recorded as doubtful. Reference to Table II will show that at a dilution of 1 : 10,000 typical reactions were regularly obtained in naturally infected persons, provided a suitable interval of time had elapsed since the ingestion of the infected

pork. Further evidence of the non-specific character of the results obtained in man in Colombia and in pigs in Panama was seen in that the reaction was progressively less severe with the increasing dilution and departed further from the typical picture in those individuals which reacted at dilutions greater than the minimal. In the known trichinous individuals, the reactions were less severe with the smaller doses of antigen, but they were nevertheless characteristic and typical. There was thus an optimum concentration at which the antigen was selective, giving reactions in trichinous and failing to give results in non-trichinous individuals.

Positive precipitin tests which may be regarded as anomalous were obtained in twenty-seven out of the 115 human subjects tested in Colombia. These were more or less proportionately divided between the individuals giving positive skin reactions and those that did not react; for example, in the first forty tested (Table I) seven positive precipitin reactions in twenty-eight positive reactors and three positive precipitin reactions in twelve non-reactors to the skin test were obtained. The occurrence of positive precipitin reactions was similarly distributed between the groups yielding positive and negative readings to the skin test among the seventy-five persons included in the later series of observations. That these results are non-specific in type is indicated by the fact that there was no correlation between the occurrence of skin reactions and the occurrence of precipitins. A further indication of the non-specificity of the reactions was the production in nine of the twenty-seven cases of a flocculent precipitate in the control tube to which only the extracting fluid (Coca's solution), used in the preparation of the antigen, was added to the serum to be tested. The character of the precipitate obtained in these twenty-seven cases differed markedly from that of the precipitate obtained in the specific reactions. With the technique employed a ring of varying thickness, depending on the concentration of the antigen in the test fluid and of the antibodies in the serum tested, is formed at the interphase of the two fluids. The ring is of equal opacity throughout. In the non-specific anomalous reactions encountered the precipitate is much denser and usually floccular and easily differentiated from the typical specific ring. As mentioned above, the occurrence of a reaction of this type in a case of mercury poisoning gave a clue to the possible significance of the results obtained. Corroboration of the suspicion of the chemical nature of the phenomenon appeared to be brought out by the fact that in twenty-three of the twenty-seven cases (see Table III) a history of recent medication was furnished. It was also possible in one instance to perform a test on the serum of an individual before and after treatment with quinine. The sample taken after quininisation gave the flocculent precipitate, but that obtained before the patient received treatment gave no reaction either with the antigen or with the extracting fluid. Similar results were obtained in an individual from whom blood samples were obtained before and after the administration of novarsenobillon. We may thus assume that the precipitates which were obtained were a result of the interaction of the various chemicals

brought together in appropriate concentration, or, alternatively, of the action of some product of the chemical reaction on the proteins present in the serum and antigen mixture. No non-specific or anomalous precipitin reactions were encountered in the sera of laboratory animals or hogs during the course of these studies. No "false positive" precipitin reactions other than those encountered in the Colombian series are recorded in our observations.

As already noted above, neither skin reactions nor non-specific precipitin reactions referable to intestinal parasites were encountered and both tests were found to be highly specific when used under suitable conditions. The extreme delicacy of the tests is particularly well illustrated in the case of the hog in the partial survey of the herd incriminated as the source of human infections. This animal which gave positive reactions to both tests was shown to be infected by recovery of the larvae by digestion of a liberal amount of muscle after a prolonged and laborious microscopic search of numerous samples had proved abortive. It is unfortunate that, through accidental oversight, muscles of the other hogs of this group which were slaughtered were not submitted to us for examination. However, no larvae were found in the musculature examined in the local, Colombian or Panamanian series of pigs which failed to give reactions to either or both of the tests. The apparent failures in two cases in man (Nos.101 and 111, Table II) were probably due to an insufficient interval of time before testing in the former and the use of too high a dilution of the antigen in the latter.

Though we have had but limited experience in the application of these tests our results indicate they may be of considerable diagnostic value in the detection of trichinosis in man or in swine. In the skin test the use of an appropriate dilution obviates non-specific reactions obtained with more concentrated preparations of the antigen. The precipitin test gave no non-specific reactions in porcine subjects and the majority of such reactions obtained in man were clearly anomalous and attributable to the medication administered before the individuals were bled for serum. Another important feature to be taken into consideration is the time factor, and positive skin reactions cannot be expected to be regularly met with before the sixteenth day after infection in man or certainly not before the tenth or eleventh day in swine. Precipitins were not demonstrated in the sera of hogs before the thirty-fifth day of infection and, though positive reactions were obtained earlier in man, such results are not regularly to be expected before the lapse of an interval of time considerably less than 5 weeks from the ingestion of the infected pork. It is expected that the tests will be of value in arriving at a correct diagnosis in human cases of some standing where the clinical picture is not clear or may be confusing, and where the course of the disease is atypical. The tests may also have some value in eliminating trichinosis in cases where myalgia, possibly associated with an otherwise unexplained eosinophilia, is the most prominent symptomatological feature. An erroneous diagnosis may, however, be arrived at in cases where there has been an earlier trichinosis, but in which the muscular pain and

other symptoms necessitating medical attention are due to some other cause. There is no evidence available to show how long after the primary trichinous infection positive reactions may be obtained. In the present work the oldest cases studied were of 6 months' standing and in these the reactions obtained were as clear-cut as those obtained in cases in which the duration was much shorter. The skin test should be of considerable value in the detection of trichinous hogs at slaughter-houses as it is extremely delicate, easily applied, and readings can be made within less than half an hour. The test can probably be made on large numbers of animals without much modification of the procedure prevailing at abattoirs and the meat of the reactors need not be destroyed, but could be diverted to the manufacture of canned or cured products, from the consumption of which there would be no danger of giving rise to human infections.

The present studies have shown that under certain conditions, and with the proper safeguards, the precipitin and skin tests furnish an accurate and reliable means of detecting trichinosis in human and porcine subjects provided the infection is not of too recent acquisition. Taliaferro (1929) adequately discusses the earlier work on precipitin and skin tests in helminth infections, but some of the more recent work merits consideration. Schwartz, McIntosh and Mitchell (1930) reported that pigs giving no skin reactions to injection of trichina extracts may become sensitised to those extracts by experimental *Trichinella spiralis* infection. In only nine out of 486 animals subjected to a skin test were reactions observed which were indistinguishable from those obtained in experimentally infected hogs. Two of these were shown to be trichinous and only one other animal which gave a moderate reaction proved to be infected. In all 127 failed to give any reaction. These findings appear seriously to conflict with those obtained by us. The authors give no indication of the technique used or of the dilution of antigen employed and, guided by our experience, we believe their discordant results are to be attributed to the use of a greater concentration of antigen than that dilution which gave such clear cut results in our hands.

Hunter (1931) and Stoll (1929) reported attempted application of the precipitin test in the detection of human trichinous infections. In Stoll's cases Bachman obtained positive precipitin reactions in samples of serum from three cases. Hunter observed positive results in four out of six cases tested. In the remaining two cases a flocculent precipitate was obtained which the author appeared to think may be an indication of extremely strong positive reactions. It is, however, probable that they may closely correspond to those observed by us in the sera of patients who had received medication.

Recent work on immunological reactions in other helminth infections has been reported by Stoll and Nelson (1930), Stumberg (1930), Taliaferro, Hoffman and Cook (1928), Coventry and Taliaferro (1928), and by Coventry (1929). Working with antigen prepared from *Ancylostoma caninum*, Stumberg was able to show that rabbits responded to injections of the material by the formation

of precipitin and complement-fixing antibodies. No antibodies were demonstrable in infected or injected dogs which, however, showed a high non-specific complement-fixation titre before injection or infection. Coventry and Taliaferro found no correlation between the occurrence of *Ascaris* and the precipitin and skin reactions obtained with *Ascaris* extracts. Slight rearrangement of the figures in the table given by these authors, however, shows that of the fifty-three individuals who gave positive precipitin reactions, forty-three (including doubtful reactors) were also positive by the skin test; thirty positive by the skin test gave negative precipitin reactions; ten negative by the skin test gave positive precipitin reactions; and the remaining seven were negative by both tests. The authors themselves intimate that a higher number of positive skin reactions would have been obtained had the intradermal method been used in making the test instead of the scratch method. This would have reduced the number of individuals giving positive precipitin but negative skin reactions. Furthermore, it seems probable to us that if a higher dilution of the test fluid had been used there would have been a considerable reduction in the number of individuals giving positive skin reactions associated with negative precipitin tests. There would thus be a high degree of correlation between the precipitin and skin tests, and it would still leave considerable discrepancy between the immunological and parasitological findings. Fülleborn's (1926) contention with regard to the persistence of sensitivity to helminth proteins has not been disposed of by these authors and it appears essential to us that studies of this nature, if expected to furnish conclusive results, should be undertaken on individuals whose parasitic history is definitely known rather than in heavily parasitised communities where all the available subjects are liable to have had infections with a number of different parasites. The same may be said, mutatis mutandis, concerning the results obtained by these authors with antigen prepared from hookworms and from *Trichuris trichiura*. Coventry in outlining the methods employed by her gives an account of the preparation of her immunising and testing fluid from whole *Ascaris*, the inference being that this was the only material used. Later, however, she mentions that one rabbit had been immunised with extract of ascaris muscle and another with a similar amount of extract of ascaris eggs. It is not clear whether all the tests were made with the same material throughout or with different batches of similarly prepared material, and it is thus not possible to estimate to what extent the apparent lack of correlation of her results may be due to variations in the technique and materials used. Taliaferro, Hoffman and Cook, using the precipitin test, obtained very promising results with a lipoid-free antigen in *Schistosoma mansoni* infections. Skin tests for *Haemonchus contortus* did not yield specific reactions in the hands of Stoll and Nelson. Their published summarised account gives no indication of the methods employed, and the impression gained is that the antigen concentration used was not of sufficiently high dilution to eliminate non-specific readings.

Articles by Taliaferro and Taliaferro (1931) and by Stumberg and

Rodriguez-Molina (1931) have been published since the above was written. Taliaferro and Taliaferro discuss their findings in skin tests with extracts of infected snail livers in *Schistosoma mansoni* infections. No uninfected persons gave positive readings to the schistosome antigen, but a number of individuals gave reactions to an extract of normal snail livers and several gave reactions when tested with material prepared from other parasites. The criteria on the basis of which the reaction obtained in any given individual is to be considered positive are discussed. Emphasis is placed on the occurrence of pseudopodial extensions, or alternatively, on the size of the smoothly contoured wheals as critical features which determine whether a reaction is to be considered positive. Pseudopodial extensions were more frequently encountered by us in the non-specific reactions induced by high concentrations of the antigen than in the specific reactions obtained by means of the highly diluted antigen. In the Colombian series, pseudopodial outrunners were observed in seventeen individuals in whom non-specific reactions were recorded. Pseudopodial outrunners were never encountered in trichinous pigs, even when they were tested with the high concentration of antigen. Similarly the severest reactions were not always seen in trichinous individuals. The importance of adequate controls is stressed. A smaller amount of the antigenic material was employed in performing the tests than was used in the work of Fairley and Williams. The authors state that the smaller test dose was used in preference to the larger "since the concentration rather than the amount appears to determine the severity of the reaction." The data in support of this statement are not furnished, and since it is stated that "after some experimentation, the quantity injected routinely was 0·025 c.c. of the 0·5 per cent. extract" it is evident the authors did not seriously consider the effect of the concentration. As shown in our work, the concentration of the antigen was the all-important factor concerned in eliciting the specific and eliminating the non-specific reaction. It is true that the reactions obtained with the higher dilutions were progressively less severe the more the antigen was diluted. However, it appears to us the less severe reactions obtained with the higher dilutions are to be attributed to the decreased amount rather than to the decreased concentration of the antigenic substance. In our opinion the specificity of the reaction is dependent on the concentration and the severity of the reaction dependent on the quantity of antigen used, other factors being equal. Such considerations, however, do not take into account either the quantity or the concentration of the "reagin" or specific sensible substance present in the host tissue on which the test is carried out. Furthermore, before a final evaluation of any of the immunological reactions in helminth infections can be arrived at, it will be necessary to determine to what extent they may be influenced by factors such as the duration of the infection, the degree of infection, previous infection with the same, related, or other parasites. Also the possibility must not be lost sight of that sensitisation to one helminth protein may bring in its train sensitisation to other helminth proteins, analogous to the condition found in hay fever, where

it has been found that substances other than the primary sensitising agent may bring on attacks and, on injection, give positive skin reactions.

Stumberg and Rodriguez-Molina present a statistical analysis of the results obtained in performing skin tests on individuals infected with hookworm. These authors mention that specificity tests were made with extracts prepared from *Ascaris* and from *B. coli*, but no indication of the frequency of these reactions is given although they discuss the absence of correlation between the size of the wheals obtained with these materials and those obtained with hookworm extract. Similarly, though much is made of egg counts and worm counts so far as hookworm is concerned, the incidence of other helminth parasites is completely ignored. Correlation of small degree between wheal size and worm count and no correlation between wheal size and egg count were found. Such a result might have been predicted, since the egg and worm count at any given moment may be the result of recent infection, long-continued exposure to light infections, the persistence of an old infection, or a combination of these factors, and under such conditions it would not be surprising to find differences in the immunological response of the host. The value of a statistical treatment of data thus obtained appears doubtful, inasmuch as the validity of the test itself is questionable under the conditions in which it was applied.

SUMMARY.

The value of precipitin and intradermal tests as a means of diagnosing trichinosis in man and swine is discussed. The precipitin test appears to be reliable in detecting established trichinosis in swine, and with the conditions under which the observations were made, is highly specific. Established trichinosis in man has been invariably detected by the precipitin test, but its diagnostic value is greatly diminished by the fact that it is also positive with serum from individuals who have recently received quinine treatment for malaria. Certain other drugs appear to affect this reaction in a similar manner. The intradermal test appears to be specific for trichinosis in man and swine when high dilutions of the test antigen are used. The reaction is positive in the early stages of the disease and is of the immediate type in both of these hosts. A survey of swine has shown that the precipitin and intradermal tests are more accurate than muscle examination.

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DESCRIPTION OF PLATE VIII.

Fig. 1. Trichinosis in human subject. Typical skin reaction (left) and control (right).

Fig. 2. Trichinosis in pig. Typical skin reaction caused by (*a*) 1 : 10,000 dilution of antigen, (*b*) 1 : 100 dilution of antigen, (*c*) control, Coca's solution

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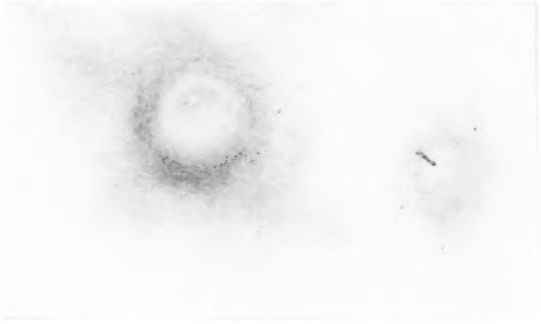

Fig. 1

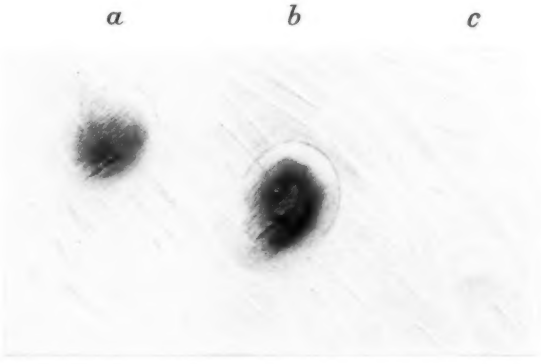

Fig. 2

STUDIES ON AVIAN CESTODES FROM CHINA.

PART I.
CESTODES FROM CHARADRIIFORM BIRDS.

By SHEN TSENG, D.Sc.

(*Zoological Laboratory, University of Neuchâtel, Switzerland.*)

(With 37 Figures in the Text.)

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Introduction.

The material upon which the present paper is based is a part of Faust's valuable collection of avian cestodes from China. It was sent by Dr E. C. Faust, formerly Professor of Parasitology in the Peking Union Medical College,

to the Zoological Laboratory, University of Neuchâtel. The collection, consisting of cestodes from charadriiform birds, was contained in 55 tubes.

The worms were collected in Peking with one exception (Mong. 4, from Kuei-Hua) in 1920–22, but for one lot (Pe 848, derived from the liver), all the worms were derived from the intestine of the birds. The following list gives the scientific names of the birds, the record number of each lot or tube of specimens and the month in parentheses when they were collected:

Aegialitis curonica (Gm.): 728, 760, 767, 841 *d* (April, May).
A. minor (Mey. et Wolf): 1462, 1476 *a*, 1496 *a* (April).
Charadrius veredus Gould: 117, 118, 752 *a*, 1416, 1452, 1474 *a*, 1482 (April).
Gallinago media (Frisch): 529, 588, 593, 748, 1466, 1487 (Jan., Feb., April).
G. megala Swinh.: 510, 517, 526, 552 *b* (January).
Lobivanellus cinereus (Blyth.): 672 *a–c*, 691 *a*, 703 *a*, 718, 1400 *b*, 1428 *a*, 1437, 1440 *c*, 1450 *b* (March, April).
Numenius arquatus (Lin.): 701 *e*, 716 *c*, 735 *c*, 1431 *d* (April).
Rhynchea capensis (Lin.): 750, 1490, 85, 107 *a*, 126 (April).
Scolopax rusticola (Vieill.): 751 (April).
Totanus calidris (Lin.): 848, 1459 (April, May).
Totanus sp.: 144 *b*, 151, 823 (May).
Tringa americana Baird: 884 *a* (May).
Tringa sp.: Mong. 4, Peking 828 (Oct. and May resp.).
Vanellus cristatus Mey. et Wolf: 1436 *a*, 1457 *b* (April).

Methods.

All the specimens studied were stained with a dilute solution of Mayer's haematoxylin (1 pt. stock sol. to 9 pts. 2 per cent. alum sol.) and were cleared finally in clove oil. Material which appeared black or deep brown was first soaked in potassium permanganate solution (1 grm. to 1000 c.c. of dist. water), then treated with oxalic acid (1 grm. to 300 c.c. dist. water), and finally stained with alum haematoxylin in the usual manner. This process was found by me to be the most satisfactory "depigmentation" method. Most of the specimens were macerated, probably owing to their having been removed from the hosts one or two days after their death. As some of the material was unsuitable for sectioning, many of the diagnoses were perforce based on *in toto* preparations, which, however, when carefully handled, appeared satisfactory for examination of the internal organs. Sections were stained with haematoxylin and eosin. All scolices were examined only in dilute glycerin.

Acknowledgments.

I desire to express my great indebtedness to Prof. Otto Fuhrmann for kindly placing the material at my disposal for investigation and I am most grateful to him for the valuable suggestions and encouragement he gave me in the course of my work.

Family DAVAINEIDAE Fuhrm.

Sub-family DAVAINEINAE Braun.

Genus **Davainea** Blanch.

Davainea himantopodis Johnston 1911 (Figs. 1–4).

Host: *Lobivanellus cinereus.*

Locality: Peking, China.

Specimens: Pe 672 *b* and 672 *c*.

Tube 672 *c* contained numerous tiny, free segments which, after being stained and mounted in balsam, proved to be all mature and gravid proglottids as figured in Fig. 2. Tube 672 *b* contained some twenty complete but very short and delicate strobilae, among which only seven or eight proglottids were mounted with scolex (Fig. 1). The posterior segments showed practically the same structure as in 762 *c*. The specific characters of both lots combined agree perfectly with the description of *D. himantopodis* Johnston, an Australian species from *Himantopus leucocephalus* Gould.

Length 0·96 mm., first proglottid 0·170 × 0·068 mm., mature 0·262 × 0·182 mm., and gravid 0·547 × 0·511 mm. Scolex suborbicular 0·204 mm. wide, suckers rounded 0·056 mm. in diam., situated in anterior portion of scolex (according to Johnston, around margin of the former there is a row of small hooks, 0·005 mm. long, which are not seen in Chinese specimens). Rostellum 0·060 mm. in diam. on apex of scolex, carrying 48 (50 ?) hooks (Fig. 3) 0·0117 mm. long, hammer-shaped, arranged in two rows (Fig. 4). Rostellar sac conicoid, extending backward to posterior side of suckers. Neck absent or present. Genital anlage (primordium) beginning immediately at 1st segment, uterus well developed in free segment which falls off after 7th segment. Genital pores regularly alternate, at anterior third of proglottis margin, genital atrium deep. Testes 40–56 mm. in diam., only four in number, three of which behind ovary, the fourth anterolateral to it on the aporal side. Vas deferens wide, much coiled behind internal end of cirrus sac. Cirrus sac large, 0·12 × 0·24 mm. directed anteromedially two-thirds across segment. Everted cirrus armed at the free end with long bristles and delicate spines at its base. Ovary consisting of two lateral rounded lobes, the whole measuring 0·128 × 0·060–0·053 mm. Vitelline gland, 0·064 × 0·024 mm., placed at hilus of the latter. Vagina wide, comparatively short, constricting before receptaculum seminis, globular sac of 0·032 mm. in diam. situated dorsal to anterolateral corner of ovary. Excretory vessels not seen. No persistent uterus. Onchospheres 0·028 mm. in diam., single in parenchyma.

With the exception of a slight difference in the form and length of hooks, this cestode is identical with Johnston's species from Australian birds. It may be noted that when Prof. Faust's collection of avian cestodes from China is fully worked out, it will undoubtedly throw some new light on the distribution of tapeworms as indicated by the present species.

Family DILEPIDIDAE Fuhrm.

(*a*) Sub-family DILEPIDINAE Fuhrm.

Dilepis sp. (Figs. 5–6).

Host: *Tringa* sp.

Locality: Kuei-Hua, China.

Specimen: Mong. 4.

Length 25 mm., breadth 2 mm. Scolex (Fig. 5) 0·399 mm. in width, bearing suckers of 0·114–0·136 mm. in diam. at its anterior margin; rostellum 0·262 mm. wide; hooks absent. Young segment 0·408 × 0·051 mm., mature (Fig. 6) 0·935 × 0·340 mm., gravid 1·6 × 0·850 mm. Genital pore unilateral, at anterior third of proglottis margin. Cirrus pouch small and short, 0·228 mm. long, approximating only longitudinal excretory vessels. Vas deferens fine, much coiled, occupying anterior space between two ovarial lobes. Ovary tubular, of two wings, aporal much larger than poral, widely expanded 0·456 × 0·125 mm. Vitelline gland, of variable form 0·171 mm. in diam. Seminal receptacle 0·148 mm. large, in front of vitelline gland, overlaid ventrally by ovary. Vagina posteriorly parallel to cirrus pouch. Testes 40–50 in number, 0·036 mm. diam., delimited laterally by longitudinal vessels. Longitudinal excretory vessels dorsal to genital ducts, the ventral of either side connected by transverse commissure. Eggs with three layers of membranes: the external 0·084 × 0·036 mm., the middle 0·048 × 0·024 mm. and the onchospheres 0·028 × 0·020 mm., its hooklets 0·010 mm. long.

So far as I am aware, the six species of the genus *Dilepis* described from charadriiform birds are: *D. tringae* Cholod. (1913), *D. retirostris* Krabbe (1869), *D. recapta* Clerc (1906), *D. nymphoides* Clerc (1903), *D. limosa* Fuhrm. (1907), *D. odhneri* Fuhrm. (1909); none of these agrees with the specimen here described, but in the absence of rostellar hooks, it is safer to leave it unnamed.

Amoebotaenia fuhrmanni n.sp. (Figs. 7–11).

Host: *Gallinago* sp.

Locality: Nanking, China, C. H. Chang Coll.

Length 1·5 to 4·77 mm., breadth 0·23 to 0·51 mm., consisting of 17 to 21 proglottids with a very short or long neck according to the degree of construction. Scolex 0·22 mm. in diam., rostellum with an apical disc of 0·068 mm.

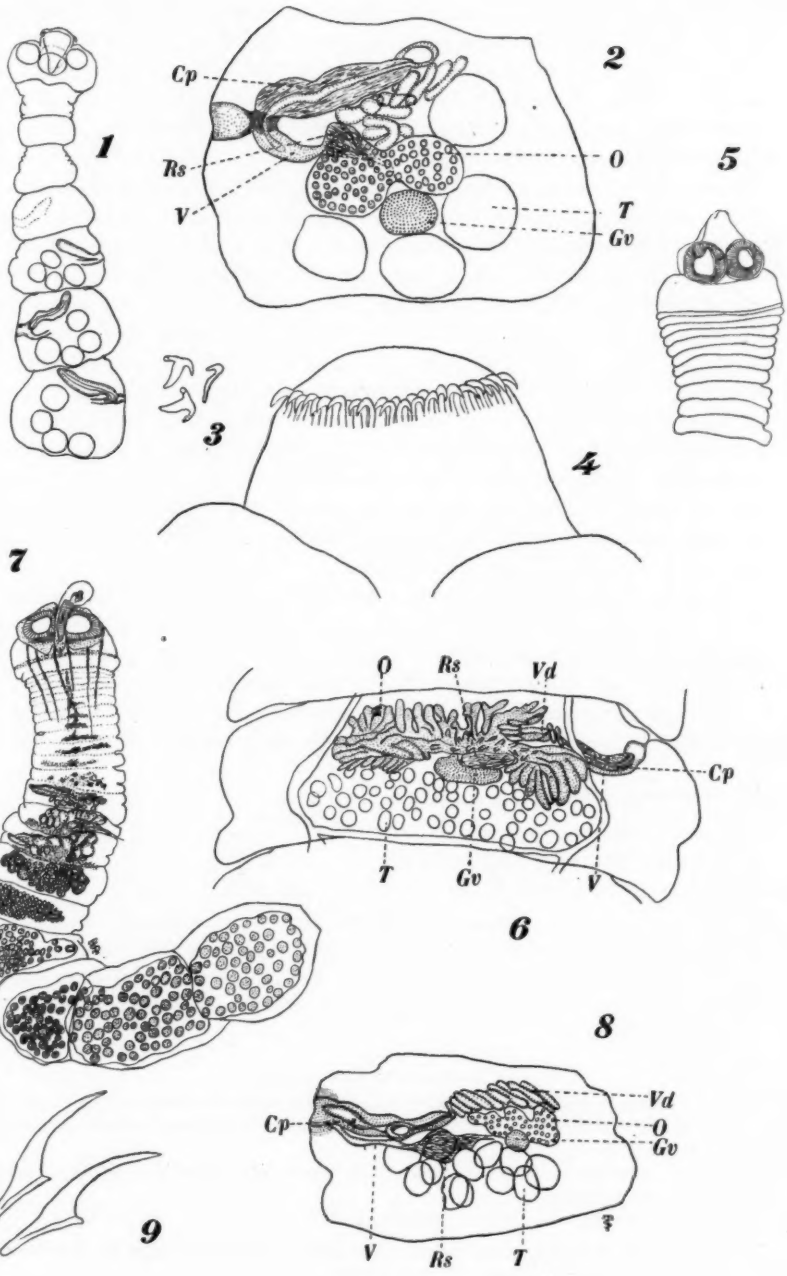
Legends to Figs. 1–9.

Figs. 1–4. *Davainea himantopodis* John. (1) Scolex and strobila showing disposition of testes in mature proglottids. (2) Mature segment. (3) Rostellar hooks. (4) Rostellum, partly extended, showing crown of hooks.

Figs. 5, 6. *Dileps* sp. (5) Scolex and part of strobila. (6) Mature segment.

Figs. 7–9. *Amoebotaenia fuhrmanni* n.sp. (7) Normal condition (balsam mount). (8) Mature proglottis. (9) Rostellar hooks.

Lettering to figures: *Cp.* Cirrus pouch; *Gv.* Vitelline gland; *O.* Ovary; *Rs.* Receptaculum seminis; *T.* Testes; *V.* Vagina; *Vd.* Vas deferens.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in diam., its double sac often extending over posterior border of suckers; 10 rostellar hooks, 0·070 mm. long; suckers rounded or oval, 0·080 mm. in diam. Genital pore regularly alternate, normally at the centre of proglottis margin. Anlage of genitalia beginning immediately behind neck. Genital organs fully developed at 14th and degenerated at 16th segment. Cirrus sac 0·044–0·052 × 0·014–0·020 mm., extending over longitudinal excretory vessels, crossing about one-third width of segment. Testes 12–16, 0·012–0·020 mm. in diam., crowded together behind female complex. Vagina posterior to cirrus pouch, extending inward from genital atrium to central globular receptaclum seminis. Ovary displaced to aporal side, of irregular shape, slightly lobed, situated between vitelline glands and vas deferens. Vitelline gland round. Uterus sac-shaped, with numerous diverticula, later filling up nearly whole segment. Eggs globular, 0·018 mm. in diam., with 2 shells; scattered loosely in parenchyme of last segments. Onchospheres, 0·014 mm. in diam.

The present species (Fig. 7) normally has a small strobila with a very short neck, which may, however, be stretched to several times its normal length. Among the specimens studied were found certain forms having a long neck, with elongated scolex (Figs. 10, 11). Therefore, the two forms might possibly be regarded as distinct species, but the difference in length of neck is simply due to the difference in the state of fixation.

Judging from all characters, such as the much less numerous but rather large rostellar hooks (10 : 0·070 mm.), the smaller testes (0·012–0·020 mm.) and the shorter cirrus sac (0·044–0·052 mm.), this tiny tapeworm is decidedly distinct from any known species of the genus Amoebotaenia.

The species is dedicated to Professor Otto Fuhrmann, University of Neuchâtel, in honour of his contributions to our knowledge of tapeworms.

Amoebotaenia pekinensis n.sp. (Figs. 12–14).

Host: *Charadrius veredus*.

Locality: Peking, China.

Specimen: Pe 1474 *a*.

Minimum length 3 mm., breadth of last proglottids 0·70–1·0 mm. × 0·374 mm. and that of young proglottids 0·42 × 0·045–0·057 mm., consisting of about 20 segments. Scolex 0·467 × 0·285 mm., rostellum 0·079 mm. in diam. with a double sac 0·2964 mm. long, extending backwards as far as the

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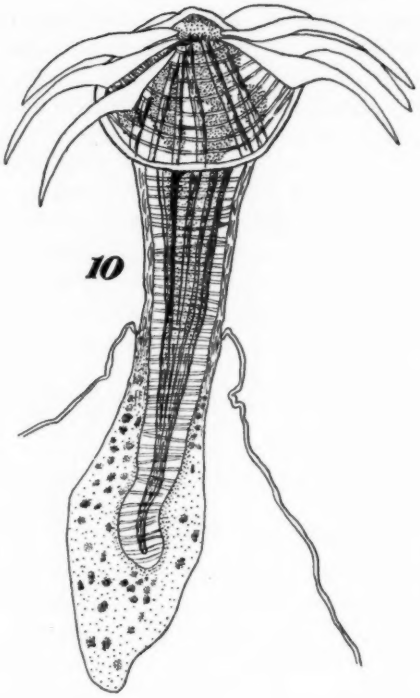
Legends to Figs. 10–15.

Figs. 10, 11. *Amoebotaenia fuhrmanni* n.sp. (10) Showing hooks of normally extended rostellum and structure of rostellar sac. (11) Scolex and part of strobila showing suckers elongated and a distinct neck.

Figs. 12–14. *Amoebotaenia pekinensis* n.sp. (12) Scolex and part of strobila. (13) Rostellar hook. (14) Mature proglottis.

Fig. 15. *Amoebotaenia vanelli* Fuhrm., rostellar hook.

Lettering to figures: *Cp.* Cirrus pouch; *Gv.* Vitelline gland; *O.* Ovary; *Rs.* Receptaculum seminis; *T.* Testes; *V.* Vagina; *Vd.* Vas deferens.



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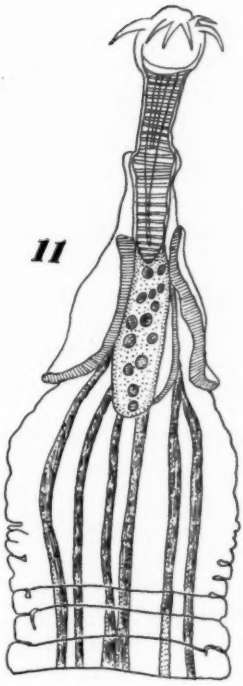

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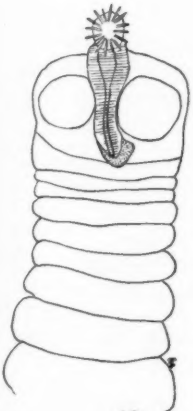

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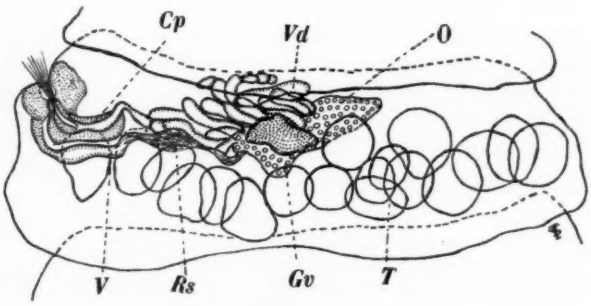

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posterior border of scolex. Rostellar hooks 16, 0·054–0·061 mm. long, arranged in one row. Neck present but very short. Proglottids broader than long. Genital pore regularly alternate, at anterior third of proglottis margin. Genital atrium well-marked, surrounded with darkly stained cells. Cirrus sac 0·040 × 0·100–0·180 mm., when extended, past longitudinal excretory vessels, penis armed with bristles. Testes 12–20, 0·060–0·080 mm. in diam., extending transversely to lateral margins in posterior half of proglottids. Vas deferens coarse, much coiled, anterolateral to female genital organs, reaching anterior border of proglottids. Vagina, a wide tube running medially posterior to cirrus sac and swelling into seminal receptacle midway between ovary and cirrus sac. Ovary an irregular mass, central, ventral to vitelline gland. Vitelline gland subtriangular, 0·080 × 0·040 mm. Eggs rounded, with two shells, 0·048 mm. in diam. (in balsam). Onchospheres 0·032 mm. in diam.

The disposition of testes and the special form of rostellar hooks justify the creation of the new species.

Amoebotaenia vanelli Fuhrm. 1907 (Figs. 15, 16).

Host: *Charadrius veredus.*

Locality: Peking, China.

Specimens: Pe 1482, 752 *a* and 117.

Length 3–5 mm., breadth 0·85 mm., consisting of 20–23 segments, all broader than long. Scolex 0·16 mm. in diam., suckers 0·102–0·136 mm. diam., rostellar disc 0·091 mm. diam., muscular sac 0·171 mm. long, extending posteriorly only to anterior level of suckers, suckers 0·102 mm. diam., hooks 16 in number 0·046–0·500 mm. long. Genital pore regularly alternate, genital atrium opening at junction of anterior and middle third of proglottis margin. Cirrus sac 0·100 × 0·038 mm. bending anteriorly from genital atrium. Vas deferens coarse, much coiled behind cirrus sac. Testes 12–18, 0·048–0·056 mm. diam., packing up behind female genital organs. Ovary central, large, trilobed. Vitelline gland round. Vagina running medially, posterior to cirrus sac, at level of poral lobe of ovary, forming receptaculum seminis, fusiform 0·028 × 0·060 mm.

Legends to Figs. 16–28.

Fig. 16. *Amoebotaenia vanelli* Fuhrm., mature segment.

Fig. 17. *Amoebotaenia brevicollis* Fuhrm., rostellar hook.

Figs. 18, 19. *Anomotaenia citrus* (Krabbe). (18) Scolex and part of strobila. (19) Rostellar hooks.

Figs. 20–22. *Anomotaenia stentorea* (Fröl.). (20) Scolex. (21) Rostellar hooks. (22) Egg with two elongated threads at opposite poles.

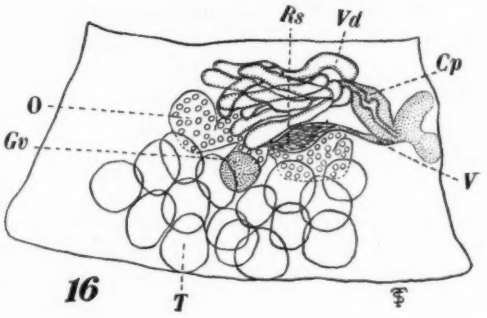
Figs. 23, 24. *Anomotaenia nymphaea* (Schrank). (23) Scolex and part of strobila. (24) Rostellar hooks.

Fig. 25. *Anomotaenia microrhyncha* (Krabbe), scolex and whole strobila.

Figs. 26, 27. *Anomotaenia* sp. (26) Mature segment. (27) Mature egg.

Fig. 28. *Icterotaenia paradoxa* (Rud.), rostellar hooks.

Lettering to figures: *Cp.* Cirrus pouch; *Gv.* Vitelline gland; *O.* Ovary; *Rs.* Receptaculum seminis; *T.* Testes; *V.* Vagina; *Vd.* Vas deferens.



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Excepting slight difference in form and size of ovary, the present specimen from *Charadrius* agrees very well with Fuhrmann's type from *Vanellus*.

Amoebotaenia brevicollis Fuhrm. 1907 (Fig. 17).

Host: *Charadrius veredus*.

Locality: Peking, China.

Specimens: Pe 1416 and 118.

Tubes 1416 and 118 contained some tiny, fragmentary, dark brown tapeworms. The process of depigmentation and staining[1], owing to the poor preservation, did not prove satisfactory for detailed anatomical study. Consequently description is incomplete: scolex subglobular 0·357 mm. wide, rostellum short with apical disc 0·136 mm. in diam. and rostellar sac 0·187 mm. long; neck present 0·323 × 0·102 mm.; young segment 0·340 × 0·034 mm., mature segment 0·374 × 0·153 mm.; testes 12–13, 0·102 mm. diam. (larger than specimen described by Fuhrmann); cirrus pouch 0·120–0·18 mm.; genital pore at centre of proglottis margin.

On certain scolices there are but few hooks, 0·064–0·066 mm. These are exactly like those of *A. brevicollis*, described in detail by Fuhrmann (1907, p. 520) and Baczynska (1914, p. 224).

According to Fuhrmann's lists of avian cestodes, only five described species occur in the genus *Amoebotaenia*. The chief differential characters of these species, including those of the Chinese forms, are given in Table I. The genital pore is regularly alternate in all seven species.

Anomotaenia citrus (Krabbe 1869) (Figs. 18, 19).

Syn.: *Choanotaenia citrus* Clerc 1903.

Hosts: *Charadrius veredus* and *Gallinago media*.

Locality: Peking, China.

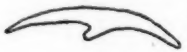
Specimens: Pe 1452, 1474 *a* and 1487.

Tube 1474 *a* contained two species of cestodes: the one is described as *Amoebotaenia pekinensis* n.sp. on p. 92 whilst the following description relates to the other.

Young tapeworms devoid of gravid segments, measuring 15 × 1 mm., consisting of 100 segments; mature segments 0·456–0·513 × 1 mm. Scolex squarish 0·408 mm. wide, rostellum 0·136 mm. diam., suckers 0·102 mm. diam., rostellar sheath extending far back of suckers. Neck present but short, distinct or not. Rostellar hooks 24 in two rows, 0·05–0·06 mm. long (Krabbe's description: 22–25, 0·054–0·062 mm. but Clerc's description: 30, 0·043–0·048 mm.). Anlage of genital organs beginning at about 15th segment. Genital pore irregularly alternate, genital atrium, when protruded, forming a cone at junction of anterior and middle third of proglottis margin. Cirrus sac extending from atrium to excretory vessels, 0·240 × 0·045 mm. Vas deferens leading from cirrus sac medially and thrown into coils nearly halfway across proglottis.

[1] See p. 92.

Species of Amoebotaenia from Birds

Species	Size of body (mm.)	No. of proglottids	Rostellar hooks: No.	Rostellar hooks: Size (mm.)	Rostellar hooks: Form	Anlagen of genital organs	Cirrus pouch in (mm.)	Testes in (mm.)	Onchospheres (mm.)	Host	Locality
A. brevicollis Fuhrm. 1907	Very small	24	16	0·059–0·061 long		Immediately behind scolex	0·24 long, cirrus armed with long, fine bristles	12–15, 0·06 diam. at posterior side of proglottis between longitudinal vessels	0·034	*Charadrius nubicus*	Egypt (Dongola)
A. frigida Meg. 1927	2·2 × 0·5	12–14	30	0·051 long, in two rows		—	0·26 × 0·064	11–15, in two rows	—	*Cypsellus apus*	Egypt
A. vanelli Fuhrm. 1907	7 × 1	25	16	0·046–0·051 long		—	0·2 long	18–20, 0·07–0·09 diam., at posterior side of segment	—	*Vanellus dongolanus* (?)	Egypt (Dongola
A. brevis v. Linst. 1884	4·2 × 1·6	12	?	0·023 ong		Beginning at 7th segment	—	—	—	*Charadrius pluvialis*, *Charadrius hiaticula*, *Squatarola squatarola*, *Aegialites hiaticola*	Europe, Africa
A. sphenoides Cohn. 1899 (Raill. 1892)	2–3·5 × 0·9–1·5	18–23	14	0·032 long		Testes seen in 1st and 2nd segment	—	12–14, against posterior border, in one row, extending to excretory canals	0·035	Fowls	South Africa, South America, Europe
A. fukrmanni mihi	1·5–4·77 × 0·23–0·51	17–21	10	0·070 long		Developing immediately behind scolex	0·044–0·052 × 0·014–0·020	12–16, 0·012–0·02 diam., behind female genital organs	0·014	*Gallinago* sp.	China (Nanking)
A. pekinensis mihi	3 × 0·7–1·0	16–20	16	0·054–0·061 long		—	0·100–0·180 × 0·020–0·040	12–20, 0·06–0·08 diam., extending laterally to both margins of segment	0·032	*Charadrius veredus*	China (Peking)

Testes 18–22 (?), 0·060–0·068 mm. diam. behind female glands. Vagina running posteriorly to cirrus sac and swelling into receptaculum seminis at internal end. Female genital glands in strobila of No. 1474 *a* not discernible, but in No. 1452 from the same host they are distinguishable. Ovary lobed, a transversely elongated sac. Vitelline gland 0·182 mm. in diam. Receptaculum seminis 0·285 mm. long overlaid ventrally by poral lobe of ovary. Shell gland stained slightly, between ovary and vitelline gland and dorsal.

Tube 1487 contained a few tiny tapeworms whose internal organs could not be clearly made out, but owing to the two crowns of hooks being similar to those of the beforementioned specimens, the position of genital pore and numerous testes, it may safely be regarded as of the same species.

The rostellar hooks are of the same size and form as in *A. citrus*, a species very imperfectly described by Krabbe (1869) and Clerc (1911) as regards the internal organs. I venture to refer our specimens tentatively to *A. citrus*, but with uncertainty.

Anomotaenia stentorea (Fröl.) (Figs. 20–22).

Syn.: *T. variabilis* Rud. 1802; *Choanotaenia variabilis* Clerc 1902.

Host: *Vanellus cristatus*.

Locality: Peking, China.

Specimen: Pe 1457 *b*.

Length 65–70 mm., breadth 2 mm. Scolex 0·296 mm. wide, with 23 hooks in two circlets, of different size: 0·040–0·045 mm., and 0·036–0·037 mm. long respectively. Suckers rounded, 0·116–0·102 mm. in diam. Rostellar sac long, extending a certain distance back of suckers. Neck prominent. Proglottis broader than long. Genital pore irregularly alternate. Cirrus sac 0·410 × 0·045 mm. Testes 35–45, 0·068–0·079 mm. in diam. Ovary tubular, large. Outer embryonal shell with two elongated threads at opposite poles of oval middle part (Fig. 23). Onchospheres 0·030 × 0·021 mm., its hooklets 0·009 mm. long.

Anomotaenia nymphaea (Schrank 1790) (Figs. 23, 24).

Syn.: *Choanotaenia nymphaea* Clerc.

Host: *Numenius arquatus*.

Locality: Peking, China.

Specimen: Pe 701 *e*.

Length 25–30 mm. (*fide* Krabbe: 75 × 1·7 mm.), breadth of young segments 0·306 mm., of mature segments 0·901 mm., and of gravid segments 1·105 mm., shape of segments inconstant, gravid segment always longer than wide. Scolex subangular, 0·342 mm. in transverse diam., sucker subcircular 0·091–0·114 mm., rostellar bulb, when retracted, 0·399 × 0·1400 mm., bearing 24 hooks 0·064–0·088 mm. long, in two circlets, rostellar sac strongly muscular, extending backward beyond scolex. Neck distinct, narrower than succeeding segments. Genital pore irregularly alternate, marginal, at anterior quarter of

proglottis margin. Cirrus sac small and short, 0·108–0·128 × 0·028–0·044 mm., hardly reaching excretory vessels, filled internally with conspicuous cirrus and coiled vas deferens which continues to poral side of ovary. Vagina opening to the exterior behind cirrus-aperture, with comparatively thick wall, leading medially to elongated receptaculum seminis, 0·056 mm. long. Ovary large, bilobed, somewhat arched, aporal lobe larger than the poral, its anterior curvature touching the posterior margin of preceding segment. Vitelline gland, 0, 1140 mm. in transverse diam., behind ovary, ventral to the latter. Testes 34–45, 0·032–0·040 mm. in diam., occupying posterior half of proglottis. Ventral vessels larger than the dorsal, lumen measuring 0·034 mm. in diam. Mature eggs not seen.

The size of hooks of this present species has been recorded by Schrank and Krabbe, but their anatomy is still obscure. By the courtesy of Prof. Otto Fuhrmann, I have been able to examine preparations of the type-specimen, and conclude that the anatomy of the Chinese species agrees on all points with that of the type.

Anomotaenia microrhyncha (Krabbe 1869) (Fig. 25).

Host: *Aegialitis curonica*.

Locality: Peking, China.

Specimen: Pe 767.

A few young small tapeworms measured 10 × 1 mm. The form of the strobila varies considerably. The normal one is shown in Fig. 25. Scolex 0·216 mm in transverse diam., rostellum 0·054 mm. in diam., sucker 0·084–0·092 mm. diam. Rostellar hooks incomplete, 0·017–0·018 mm. long, arranged in two rows. Genital pore irregularly alternate. Testes 20–25, 0·034–0·057 mm. Cirrus pouch small, 0·100 × 0·016 mm. Ovary bilobed, compact, 0·228 mm. in total width. Vitelline gland 0·148 mm. in diam. Eggs not seen.

According to the above description the present specimen has some characters similar to *A. microrhyncha*, recorded from the intestine of the ringed plover (*Aegialitis hiaticola* L.) from different localities.

Anomotaenia sp. (Figs. 26, 27).

Host: *Vanellus cristatus*.

Locality: Peking, China.

Specimen: Pe 1436 *a*.

Length 87 mm., greatest breadth 1·7 mm. Young segments immediately behind scolex measuring 0·119 × 0·017 mm. broader than long; mature segments 1·285 × 0·935 mm., posterior gravid segments 1·530 × 1·700 mm. longer than broad. Neck present, 0·510 mm. long. Scolex 0·240 mm. diam.: rostellum (in balsam) 0·0510 mm. diam., muscular sac 0·170 mm. long, reaching backward to posterior margin of scolex; hooks absent; suckers 0·170 mm. in diam. Anlage of genitalia commencing at about 82nd segment from neck. Testes numerous, about 40–50, 0·171–0·262 mm. in diam., backed up at the posterior

part of segment, delimited laterally by longitudinal excretory vessels. Ovary of complicated tubular glands, developed enormously, occupying nearly anterior two-thirds of proglottis. Vitelline gland deeply lobed, central, 0·193 × 0·182 mm. Vagina running inward from genital atrium to an oval receptaculum seminis, 0·228 × 0·114 mm., lying just in front of vitelline gland. Cirrus sac, narrow, 0·547–0·575 × 0·068–0·114 mm. hardly passing over ventral longitudinal excretory vessels. Genital atrium irregularly alternate, opening at anterior third of proglottis margin, presenting the following sequence: LLLRLLRRLLLRLLR. Fine vas deferens strongly coiled, extending immediately from internal end of cirrus sac to left-hand of receptaculum seminis. Dorsal longitudinal vessels measuring 0·684 mm. in diam., ventral vessels subequal (0·684–0·114 mm.), a transverse canal connecting the latter of either side along the posterior border of proglottis. Gravid segment filled with fusiform eggs, the first shell measuring 0·216 × 0·036 mm., the second 0·050 × 0·0252 mm.

According to the form of eggs, the present form differs from the genus *Monopylidium* or *Choanotaenia*, but in the absence of rostellar hooks it is tentatively placed under *Anomotaenia* and remains unnamed.

Icterotaenia paradoxa (Rud. 1802) (Fig. 28).

Syn.: *Choanotaenia paradoxa* Clerc 1903, *Parachoataenia paradoxa* Lühe 1910.

Host: *Aegialitis curonica*.

Locality: Peking, China.

Specimen: Pe 728.

Tube 728 contains a few fragments of small brown tapeworms. Preparations *in toto* seem insufficient for determination, although the scolex and gravid segment point to the genus *Icterotaenia*. A careful study of sections permits of the following description:

Length ?, gravid segments 0·894 × 0·935 mm., that of sexually mature segments 0·595 × 0·357 mm. Scolex 0·612 mm. wide, followed by a long neck about as wide (0·578 mm.), no distinct demarcation between scolex and neck, thus the anterior being cylindrical. Rostellum retracted, measuring at level of circlet of hooks 0·171 mm. in diam., armed with 15 ? hooks 0·086–0·088 mm. long in one row. Rostellar sac 0·442 × 0·341 mm. Suckers 0·200 mm. diam., situated on a level with anterior end of muscular sac. Genital pore opening at anterior third of proglottis margin, its atrium not distinct. Cirrus sac rather long, narrow, 0·100 × 0·012 mm., cirrus fine and straight. Vas deferens loosely coiled behind internal end of cirrus sac, forming neither internal nor external seminal vesicles. Testes in smaller number, 12–14 (on frontal section), 0·032–0·044 mm. diam. Semicircular ovary, 0·044 × 0·12 mm., situated at the middle of proglottis. Vitelline gland, 0·076 mm. wide, some distance behind ovary. Vagina posterior to cirrus sac, directed posteromedially, and at region of ovary swelling into receptaculum seminis, 0·032 × 0·048 mm. At the median end of the latter, vagina constricted at first and then again expanded. On section, the first portion of vagina lined with cilia. In gravid segment, one onchosphere

in each parenchymatous capsule, which measures 0·068 mm. diam. (in balsam).

A brief comment on this species may be made as follows: In 1850–51, Diesing, when describing *Taenia paradoxa* Rud., pointed out the special form of proglottids "articuli difformes." In 1869, Krabbe mentioned that *Taenia paradoxa* bears 14–19 hooks 0·074–0·098 mm. long (misquoted by Clerc as 0·074–0·078 mm.) and their basal part is straight with a ventral rudimentary root, as shown in his fig. 70, pl. III. The size given by Clerc for this species is much smaller, being 0·050–0·075 mm., and the form appears different from that figured by Krabbe, *i.e.* the basal part of hooks drawn by Clerc in fig. 62, pl. 10 looks rather recurved. However, he was of opinion that the deformity of proglottids in his form agrees with Diesing's description. Of the present specimen, the form and size of hooks (Fig. 28) are identical with those figured by Krabbe, but the anatomy mentioned above does not agree with Clerc's description. On account of insufficient and fragmentary material at hand, it is impossible to verify the degree of deformity of the proglottids, therefore, a further study of additional material is necessary.

Icterotaenia arquata (Clerc 1906).

Syn.: *Choanotaenia arquata* Clerc 1906.

Host: *Numenius arquatus*.

Locality: Peking, China.

Specimen: Pe 716 *c*.

Tube 716 contained a few small fragments moderately preserved, which, after careful treatment rendered a study of the internal anatomy of proglottids possible. The following account is incomplete:

Young segments slightly broader than long, 0·342 × 0·288 mm.; mature sexual segments 0·570 × 0·649 mm. Genital pore irregularly alternate. Testes 15–20, 0·028–0·057 mm., vitelline gland 0·048 mm.; ovary in two deeply lobed wings, connected by a narrow isthmus; cirrus sac short, hardly one-third the width of proglottids.

Definite determination is impossible on the incomplete material in hand, but comparing the internal structures with those of *Icterotaenia* (*Choanotaenia arquata* Clerc, recorded from the same host, the present specimen bears some similarity thereto.

(*b*) Sub-family DIPYLIDIINAE Stiles.

Monopylidium macracanthum Fuhrm. 1907 (Fig. 29).

Host: *Lobivanellus cinereus* and *Rhynchea capensis*.

Locality: Peking, China.

Specimens: Pe 1400 *b*, 107 *a* and 703.

Tube 1400 *b* contained two species of tapeworms of a *Gyrocoelia* sp. to be described later, and the smaller ones showing the characters of *Monopylidium* with strobila 20–60 × 2 mm.

Length ?, 1–2 mm. broad. Scolex 0·592 mm. in transverse diam., rostellum 0·239 mm. in diam., rostellar sac 0·364 mm. long, suckers 0·182–0·193 mm. in diam. Neck very short, 0·57 × 0·020 mm. Rostellar hooks 26 in number, of two sizes: larger 0·144–0·148 mm., smaller 0·120–0·124 mm. long, in two rows. Genital pore irregularly alternate, at anterior third of proglottis margin. Cirrus pouch 0·342 × 0·034 mm., in mature segments, or 0·228 × 0·045 mm. in gravid segments, slightly overlapping excretory vessels. Vas deferens closely coiled, partly overlaying ovary. Ovary small, 0·160 mm. wide, in front of vitelline gland, 0·079 mm. in diam. Testes 22 ? or more, 0·057 mm. in diam., in posterior half of segment. Calcareous corpuscles abundant. Eggs 0·079 mm. in diam., single in each parenchymatous capsule.

The material from *Rynchea capensis* was all fragmentary and macerated. Careful examination of the scolex and some mature segments appeared to reveal the following characters: Scolex 0·765 mm. wide, rostellum retracted, 0·306 mm. in diam., rostellar sac 0·452 mm. long, extending far from suckers 0·187–0·204 mm. in diam., neck indistinct, rostellar hooks in two rows, 0·124–0·148 mm. long, genital pore irregularly alternating, cirrus pouch 0·193–0·228 × 0·045 mm., testes ?, 0·114–0·136 mm. diam., ovary ?, vitelline gland 0·079 mm. diam.

The poor material rendered detailed study of other structures impossible. Tube 703 contained a few specimens in company with *Gyrocoelia* sp.

Monopylidium macracanthum, as fully described by Fuhrmann (1907), Mapleston and Southwell (1923) bears a double crown of long hooks which are identical with the specimens here considered; the internal anatomy shows some similarity to the description of Fuhrmann's species, but cirrus sac is a little longer (only 0·16 mm. long: *fide* Fuhrmann).

Monopylidium guiarti n.sp. (Figs. 30–32).

Host: *Aegialitis minor* and *Aegialitis curonica*.

Locality: Peking, China.

Specimens: Pe 1462, 1476 *a* and 1496 *a*.

Tubes 1462, 1476 *a* and 1496 *a* each contained one characteristic specimen of *Monopylidium guiarti*.

Legends to Figs. 29–37.

Fig. 29. *Monopylidium macracanthum* Fuhrm., rostellar hooks.

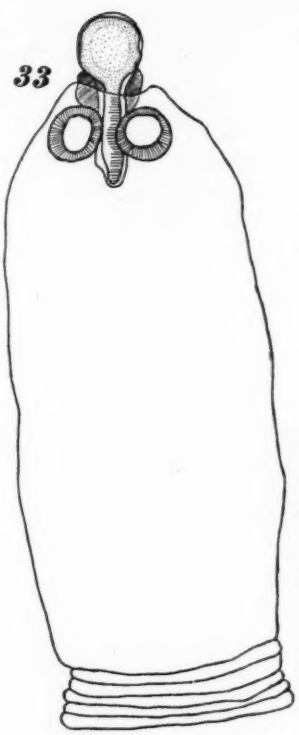
Figs. 30–32. *Monopylidium guiarti* n.sp. (30) Scolex and anterior segments of strobila. (31) Rostellar hooks. (32) Mature segment.

Figs. 33, 34. *Monopylidium* sp. (33) Scolex and neck. (34) Onchosphere surrounded with parenchyma.

Fig. 35. *Choanotaenia cingulifera* (Krabbe), isolated rostellar hook.

Figs. 36, 37. *Choanotaenia joyeuxi* n.sp. (36) Rostellar hooks. (37) Mature segment.

Lettering to figures: *Cp.* Cirrus pouch; *Gv.* Vitelline gland; *O.* Ovary; *Rs.* Receptaculum seminis; *S.* Shell gland; *T.* Testes; *V.* Vagina; *Vd.* Vas deferens.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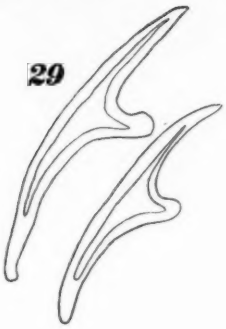
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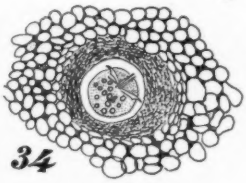
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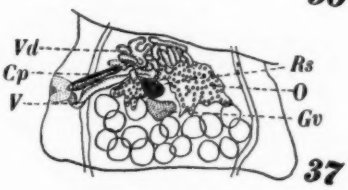
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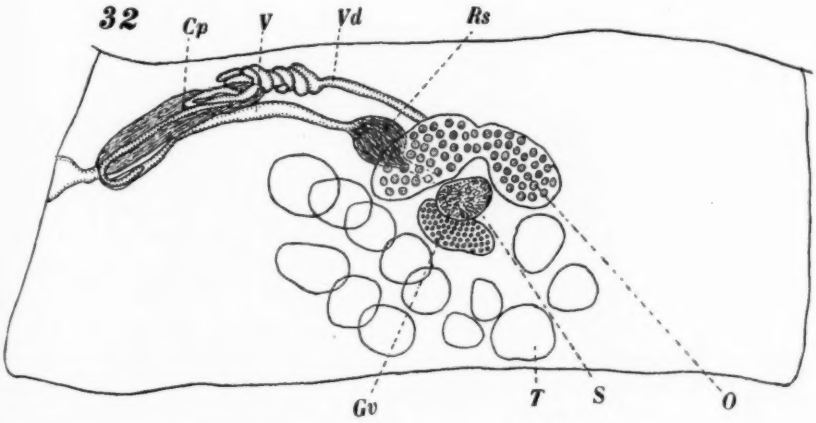
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Length 17 mm., consisting of about 100 segments, young proglottis measuring 0·408 × 0·017 mm., mature 1·105 × 0·34 mm., all broader than long, gravid 0·850 × 0·680 mm. longer than broad. Neck not very sharply demarcated. Scolex 0·627 mm. in transverse diam., rostellum 0·193 mm. diam., muscular sac 0·469 mm. long, extending backward far behind suckers, suckers 0·193 mm. diam., hooks 30, in two rows, 0·084–0·096 mm. long. Genital pore irregularly alternate, LLLLRLRLLLLRRLLL, at the anterior third of proglottis margin, no well-marked atrium. Cirrus sac 0·200–0·360 × 0·024–0·045 mm., extending medially a little past excretory vessels. Testes 12–20, 0·034–0·057 mm. behind female genital glands, sometimes extending more forward on either side of ovary. Vagina, fine and straight, posterior to cirrus sac. Receptaculum seminis 0·120 mm. long, dorsal to ovary. Ovary crescentic, central, compact, embracing rounded vitelline gland, 0·072–0·080 mm., in its posterior hilus. In gravid segment, ova scattered singly in parenchyma. Eggs about 0·048 mm. in diam.

Apart from the internal anatomy which seems different from other known species occurring in closely allied hosts, the form of the hooks is similar to that of *M. chandleri* Moghe 1925, *M. cayennense* Fuhrm. 1909, and *M. macracanthum* Fuhrm. 1907, but the number of hooks in all these species is less than 30. *M. arcticum* (*Choanotaenia arcticum*) Baylis 1919, and *M. secundum* Fuhrm. 1907 are similar to the present specimens in the number of hooks, but different in shape and length. Therefore we regard the form here considered as a new species which is named in honour of Dr J. Guiart, Professor of Parasitology of the University of Lyon, France.

Monopylidium sp. (Figs. 33, 34).

Host: *Lobivanellus cinereus*.
Locality: Peking, China.
Specimen: Pe 672 *a*.

Tube 672 *a* contains one large tapeworm devoid of rostellar hooks, but judging from the shape of eggs, the number and disposition of testes and its irregularly alternate genital pore, it belongs to the genus *Monopylidium*.

Length 110 mm., greatest breadth 4 mm. Scolex not differentiated from the neck (see Fig. 33). Rostellum 0·289 mm. diam., suckers comparatively small, 0·221 mm. diam., rostellar sac 0·510 mm. long, extending backward over suckers. Young segment immediately behind neck measuring 0·952 × 0·119 mm.; mature segments 2·3 × 0·68 mm., all broader than long, last gravid segment squarish, 2·0 × 2·0 mm. Genital pore irregularly alternate. Cirrus pouch 0·456 × 0·045 mm. Vagina 0·002 mm. wide. Genital ducts passing dorsally to longitudinal ducts. Vas deferens thrown into complex loops internal to cirrus pouch and anterior to seminal receptacle which measures 0·1254 mm. in transverse diam. Testes about 60 in number, 0·068–0·078 mm. in diam., posterior and lateral to female organs. Ovary and vitelline gland not

imperfectly seen even in sections. Onchospheres 0·05 mm. diam., one in each capsule (0·072 mm. diam.) densely surrounded by parenchyma (Fig. 35).

The specimen differs from other known species of the genus, but in the absence of rostellar hooks and the shape of female glands not being clearly established, the species is undeterminable.

Choanotaenia cingulifera (Krabbe 1869) (Fig. 35).

Syn.: *Monopylidium cingulifera* Clerc 1903.

Host: *Totanus* sp.

Locality: Peking, China.

Specimen: Pe 823 *a*.

A very fragmentary specimen, length?, breadth? (*fide* Krabbe, 100 × 1·0 mm.; Clerc, 80–120 × 1·0 mm.). Mature segment 0·459 × 0·314 mm., gravid segment 0·680 × 0·391 mm., young segment 0·084 × 0·08 mm. Scolex 0·124 mm. diam., rostellum short, 0·060 mm. diam., suckers 0·036 mm. Rostellar hooks 40, 0·005 mm. long, in one row, somewhat similar to those of Davainea in shape (length appears very variable, *fide* Krabbe, 0·004–0·005 mm.; Clerc, 0·007 mm.; Skrjabin, 0·007 mm.). Genital pore irregularly alternate, in front of centre of proglottis margin. Cirrus pouch short, pyriform, 0·20–0·68 × 0·128 mm. Testes 40, 0·016–0·032 mm. diam., occupying posterior, anterior and aporal side of female organs. Ovary not discernible, appearing to approach poral side. Seminal receptacle 0·040–0·060 × 0·032–0·036 mm. Vitelline gland 0·040 mm. diam., shell gland not seen. No ripe eggs, but in gravid segment a single egg in capsules, which fill up all the space between longitudinal excretory vessels as figured by Skrjabin (1914).

The specimen agrees in most specific characters with Clerc's (1903) and Skrjabin's (1914) descriptions of *M. cingulifera*. Unfortunately, no further figures of rostellar hooks are to be found. The hooks as figured by Krabbe (1869, fig. 59, pl. III) seem to show a little difference (see Fig. 36).

Choanotaenia joyeuxi n.sp. (Figs. 36, 37).

Host: *Scolopax rusticola*.

Locality: Peking, China.

Specimen: Pe 751.

Tube 751 contains 2 species, one a *Haploparaxis* sp., the other is here described as new.

Length 45 mm., mature segments 0·65 mm. wide, broader than long, young segments behind neck 0·228 × 0·0228 mm. Scolex 0·353 × 0·307 mm., rostellum 0·080 mm. in diam., rostellar hooks only ten, in a single row, 0·0234–0·025 mm. long, rostellar sheath extending backward before posterior margin of suckers, suckers 0·148–0·150 × 0·182–0·205 mm. Neck present, about 0·228 mm. long. Genital pore irregularly alternate, at centre of proglottis margin. Cirrus sac 0·028 × 0·102–0·188 mm., straight, running obliquely

from atrium to anterior border of proglottis. No internal or external seminal vesicle. Vas deferens much coiled anteromedially to cirrus sac. Testes 14–18, 0·034–0·045 mm. diam., delimited laterally by excretory vessels at posterior half of segment. Vagina 0·020–0·032 × 0·200 mm., larger and longer than cirrus sac. At internal extremity of vagina, forming oval receptaculum seminis, 0·068 × 0·08 mm. at level of ovary. Ovary of two lobes, antiporal larger than poral, both slightly lobulate. Vitelline gland 0·684 × 0·057 mm., central, in posterior concavity of ovary. Mature eggs not seen. No persistent uterus.

The rostellar hooks are shaped much like those of *Icterotaenia decacantha* Fuhrm. (*Choanotaenia decacantha* Fuhrm. 1913), but a little longer, and the internal anatomy is very different. The specimen is described as a new species of *Choanotaenia*. It is named after Dr Ch. Joyeux, Professor of Parasitology, University of Marseilles, France.

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THE PRIMARY LARVAE OF THREE OPHIONINE ICHNEUMONIDS, PARASITIC ON *RHYACIONIA BUOLIANA*.

By W. H. THORPE, M.A., Ph.D.

Imperial Institute of Entomology.

(With Plate IX, containing Figs. 1–11.)

In a previous paper concerning the numerous species of insect parasites associated with the Pine Shoot Moth, *Rhyacionia buoliana* Schiff., the mature larvae of the more important Hymenoptera were described in detail, special attention being paid to the mouth parts and tentorial structures which were found, in many cases, to provide valuable characters for distinguishing the species.

As far as the primary larvae are concerned the most interesting forms found attacking *R. buoliana* are those belonging to the Ichneumonine group Ophioninae. The adults and mature larvae of the following five species were obtained and were described in the above paper:

Cremastus interruptor Grav. — *Omorgus borealis* Zett.
Eulimneria rufifemur Thoms. — *Omorgus ensator* Grav.
Omorgus mutabilis Holmg.

Owing, however, to the uncertainty as to the identity of the several primary larvae no description of these was published at the time. Since then the life history of the commonest form, *Cremastus interruptor*, has been followed right through. A first-stage larva, apparently that of *Eulimneria rufifemur*, which, as regards abundance, takes third place on the list, has also been obtained; but as it seems indistinguishable in all reliable characters from that of *Eulimneria crassifemur* (Thoms.), studied by Thompson and Parker, it is not described in detail in this paper, the head alone being figured (Fig. 6).

The other remarkable larva described here is second in abundance to *Cremastus*. Although all stages have not been isolated there seems no doubt that it can belong only to *Omorgus mutabilis*. Of the three species of this genus, *mutabilis* is the common one, being second numerically to *Cremastus*. The remaining two species are decidedly uncommon and their first stage larvae are not known.

Omorgus mutabilis Holmg.

The primary larva (Fig. 1) is 2·5–2·8 mm. long. It is of the regular ichneumoniform type, approximately cylindrical, without abdominal spines or tubercles. The head capsule is slightly sclerotised, there are thirteen body

segments, the last being produced into a slender, tapering tail which is about three-quarters of the length of the body itself. The drawing shows the larva after emergence and before much food has been taken. At this time the body segments are considerably longer than broad, but towards the end of the first stadium they are much swollen and lose their crenate outline.

Apart from the great length of the tail the most striking feature of the larva is the presence of a pair of large, tusk-like sense organs projecting downwards and backwards from the posterior margin of the head capsule. They are not sclerotised and are semi-transparent. They do not appear capable of any extensive independent movement. On the anterior edge of each of these organs, not far from the tip, is a minute sense papilla. Anteriorly, just behind the mouth, there is another pair of sensoria of the usual type, placed nearer the mid-ventral line. From the prominence of these "tusks" and from their position, one might conclude at first sight that they represented the labial palpi. This, however, does not appear to be the case, for examination of the mouth region shows the presence of labial and maxillary plates with their sensillae closely similar to those described for the first stage of *E. crassifemur*, and corresponding, in general plan, to the mouth parts of mature Ichneumonid larvae. It seems, therefore, that these conspicuous tentacle-like structures merely represent one of the four pairs of hair-like sense organs usually present on the ventral surface of the head of primary Ichneumonid larvae and are not true appendages.

The mouth parts are as follows. The epipharyngeal region bears five pairs of sensillae. There is a pair of sharply pointed mandibles. The heart-shaped labial plate bears two pairs of sensillae and the lateral maxillary plates one pair. Underlying the latter, a pair of dark, sharply pointed rods can be distinguished. There is also a pair of sensillae at the base of each anterior mandibular condyle. The shape of the capsule itself, and the position of the remaining sensillae, can be seen from the illustrations (Figs. 7 and 8).

The tracheal system is of the usual closed type; the secondary thoracic tracheal trunk being present. Details of the tracheal system in the head and thorax are shown in Fig. 7. The great length of the tail is remarkable. As far as I am aware no other known larva has this organ so well developed. In most species, hitherto described, it is not more than about one-third of the length of the body. Its cavity is continuous with that of the body and the general structure is similar to that described by Thompson and Parker in *Eulimneria crassifemur*.

In neither of the larvae here described is there anything comparable to the bundles of tracheids, and the large air cavities separated by protoplasmic walls, described by Tothill in *Campoplex pilosulus*; and my observations serve to confirm the conclusion of Thompson and Parker that these were merely artefacts. *O. mutabilis* does, however, differ from *E. crassifemur* in that each main tracheal trunk does not actually terminate in the lobe of fat body, but after passing through it divides into two very fine branches which can be traced for

about one-third or one-half the length of the tail. In many larvae a small third branch can also be detected (Fig. 4). The lumen of the tail is, of course, filled with blood, but there is no circulation of the fluid and the tail is not capable of independent movement. The chitinous cuticle of the tail region is not noticeably thinner than elsewhere on the body surface.

The larva is remarkable for its activity. On dissecting it from its host it lashes its body to and fro and it will remain alive and active in Ringer's solution for twelve hours or more.

Only one larva can reach maturity in a single host.

Cremastus interruptor Grav.

Total length (young first stage larva) 2·6 mm. A detailed description of this larva is not necessary as the illustrations (Figs. 2, 3, 5, and 9–11) show the chief characters. The striking features are the very heavily sclerotised and pigmented head capsule and the two ridges encircling each of the post-cephalic segments.

In place of the tusk-like organs of *O. mutabilis*, the two median pairs of ventral sensillae are mounted on heavily pigmented, chitinous knobs. The mouth parts are similar to those of *Omorgus*, but the mandibles are small and the labial plate is small and ill defined. Owing to the dark colour of the head capsule the sensillae are difficult to see, and I can only distinguish the following: epipharyngeal—four pairs; maxillary—one pair; labial—one pair.

The segmental ridges mentioned above are visible only in the young larva; when fully fed they are smoothed out, but they reappear in the cast skin directly the tension is relieved.

The tail varies considerably in length, but in some cases is practically as long as the body itself. The cuticle at the base of the tail is markedly thicker than that covering the general body surface but is thinner towards the tip.

The tracheal system resembles that of *O. mutabilis* save that there is a greater supply of tracheal branches to the head (Fig. 10), and that posteriorly each tracheal trunk, having passed through the fat body in the tail, divides into three very fine branches which extend nearly two-thirds of its length (Fig. 5). Various attempts were made to see whether, by extending the column of air in these branches, they could be followed still further. Larvae were punctured and immersed in hypertonic solutions of sodium chloride and potassium lactate, but without effect. Perhaps this is not surprising however, for in these parasite larvae it is not usual to find an abrupt termination of the air column such as Wigglesworth (1930) has described in the tracheoles of *Stegomyia*. Blunt terminations are occasionally discernible, particularly in larvae that have been injured, but they appear to be quite normal.

The second stage larva, as is usual, lacks the heavily chitinised head capsule and the tail is very greatly reduced.

Cremastus interruptor is a very common species. As many as four or five larvae may be found together in a single host, but only one reaches maturity; all the others succumb early in the first stage. When found in company with the Braconid, *Orgilus obscurator*, the latter is invariably destroyed.

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EXPLANATION OF PLATE IX.

Fig. 1. *Omorgus mutabilis.* Early first stage larva.

Fig. 2. *Cremastus interruptor.* Early first stage larva.

Fig. 3. *Cremastus interruptor.* Early first stage larva. Tracheal system of right side.

Fig. 4. *Omorgus mutabilis.* Diagrammatic longitudinal section of tail of first stage larva, showing mode of ending of tracheal trunk of right side.

Fig. 5. *Cremastus interruptor.* Diagrammatic longitudinal section of tail of first stage larva, showing mode of ending of tracheal trunk of right side.

Fig. 6. *Eulimneria rufifemur.* Head of first stage larva, lateral view.

Fig. 7. *Omorgus mutabilis.* Head of first stage larva, lateral view; showing tracheal system of right side only.

Fig. 8. *Omorgus mutabilis.* Head of first stage larva, ventral view.

Fig. 9. *Cremastus interruptor.* Head of first stage larva, lateral view.

Fig. 10. *Cremastus interruptor.* Head of first stage larva, lateral view; showing tracheal system of right side only.

Fig. 11. *Cremastus interruptor.* Head of first stage larva, ventral view.

Explanation of lettering on figures: *a*, anus; *ac*, anterior commissure; *c*, cuticle; *ep*, epipharyngeal region; *fb*, fat body; *hf*, hypodermal fibres; *hg*, hind gut; *lb*, labial plate; *mx*, maxillary plate; *slt*, secondary lateral trunk; *t*, trachae; 1 and 2, anterior and posterior ventral sensillae.

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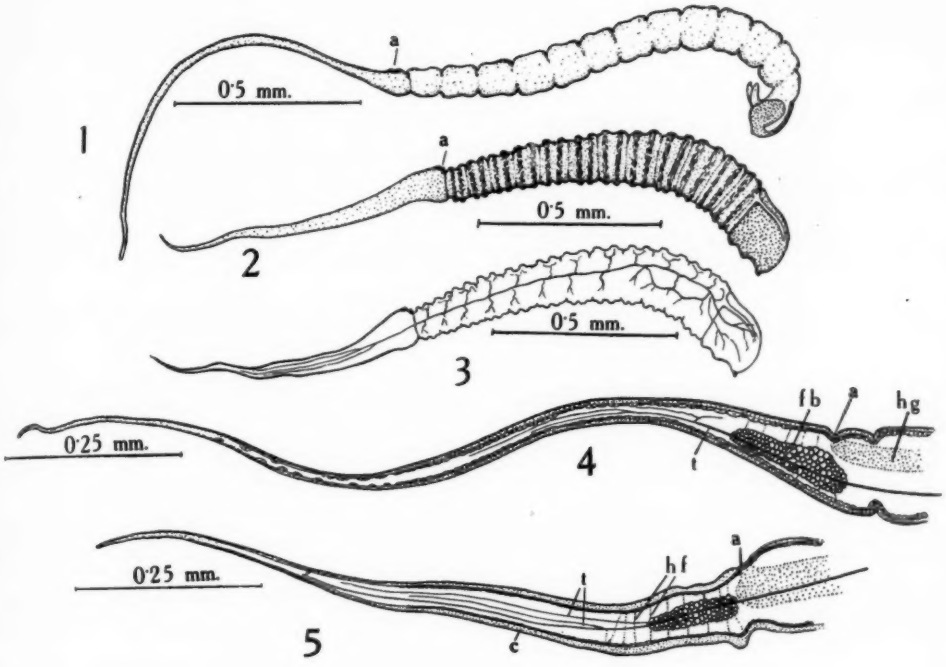
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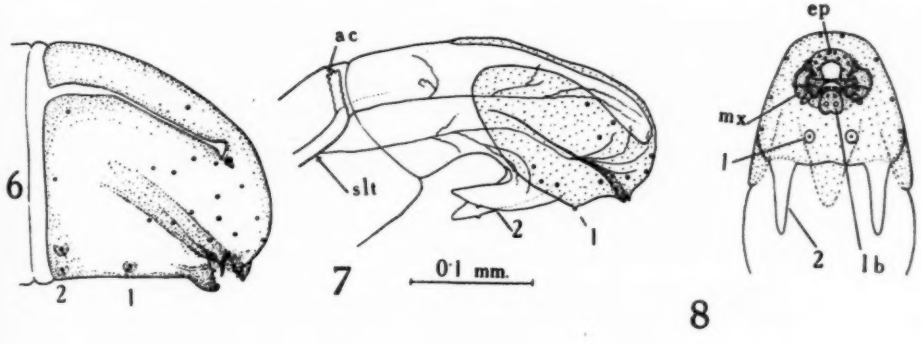
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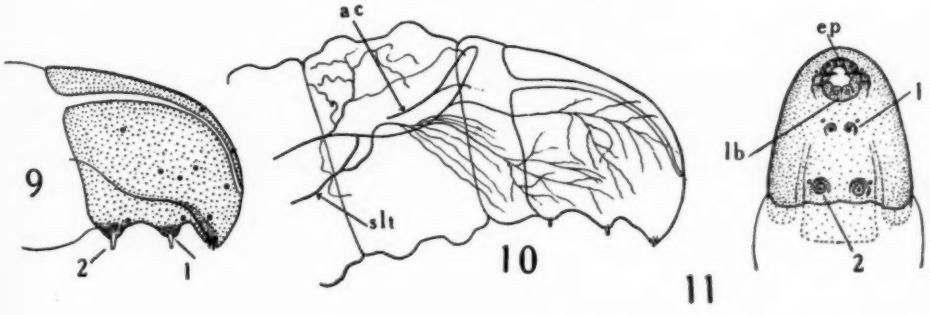
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THE LARVAL INSTARS OF *ORTHOPODOMYIA PULCHRIPALPIS* ROND. (DIPTERA NEMATOCERA).

By P. TATE.

From the Molteno Institute for Research in Parasitology, University of Cambridge.

(With Plate X, containing Figs. 1–27, and Fig. I in the Text.)[1]

Orthopodomyia pulchripalpis was first recorded in Britain by MacGregor (1919), who found it, in association with *Anopheles plumbeus* Steph. and *Aëdes geniculatus* (Olivier), in a water-filled hole in a beech tree in Epping Forest. He described it as a new species, *O. albionensis*; but later Edwards (1922) showed it to be synonymous with *O. pulchripalpis* Rond. Keilin (1927, 1927*a*) found larvae of *O. pulchripalpis* in a water-filled hole in a horse-chestnut tree in Cambridge, thus establishing a second locality for this mosquito in Britain. Beattie and Howland (1929) recorded it from water-filled holes in beech trees at Burnham Beeches, Buckinghamshire. In each case it was found in association with *Anopheles plumbeus* and *Aëdes geniculatus*.

MacGregor (1919), besides describing the imagines, gave a brief description of the 4th instar larva and of the pupa, and states that two larvae sent to F. W. Edwards were said by the latter to be in the 3rd instar and to differ from the 4th instar in colour and in not having plates on the dorsum of the posterior abdominal segments.

In spite of the later history of the horse-chestnut tree reservoir discovered by Keilin (to appear in *Parasitology*), since 1927 it has continued to yield abundant material of *Orthopodomyia*, and has enabled me to study the four larval instars. I have not succeeded in finding eggs of *O. pulchripalpis*, but Howard, Dyar and Knab (1912, p. 890) state that *O. signifer* lays its eggs singly close to the water line on the sides of the water-containing cavity and describe the eggs as subcylindrical, black, and covered with a gelatinous membrane which overlaps considerably on each side where it is much ridged transversely.

In the horse-chestnut reservoir larvae of *O. pulchripalpis* are usually present all the year round and are especially abundant in July and August. The 4th instar larvae may over-winter in the reservoir, and even if these larvae are brought indoors and kept in a warm room they will not pupate until the following spring. Although the imagines live for several weeks in captivity if fed on raisins or apple they do not breed. The females, in captivity, would not bite man, birds, toads, or invertebrates such as earthworms and slugs.

[1] Figures occurring on plates bear Arabic numbers, whilst text-figures bear Roman numbers.

In describing the larvae the terminology of Lang (1920) is used for the hairs of the head capsule as far as possible; mostly the terminology of the mouth-parts is that used by Puri (1931) for Anopheline larvae; and the nomenclature of the different types of hairs is that introduced by Nuttall and Shipley (1901) and extended by Wesché (1910). However, I call hairs which have a simple basal shaft and then split into branches "branched hairs" in preference to the terms "plumes" or "tufts" of other workers; and when the branches are numerous and lie in one place I call them "fan-like" hairs.

4TH INSTAR LARVA.

The mature 4th instar larva is about 10 mm. long and 2 mm. wide across the thorax. The thorax is broad, roughly twice as wide as the head; and the abdomen, half as wide as the thorax in front, tapers slightly posteriorly. Usually the larva has a marked red colour, which is due to bands of pigment-containing tissue encircling the segments beneath the cuticle. The thorax shows three broad, irregular, anastomosing pigment bands. In the abdomen the pigment bands are narrow in the anterior segments and gradually increase in width in the posterior segments.

Head. The head is brown, well chitinised, and sub-quadrate in shape with the posterior margin rounded. It is broadest immediately behind the eyes which are placed midway between the bases of the antennae and the occipital margin. The fronto-clypeus is broadly rounded anteriorly and narrows posteriorly to end in a rounded margin dorsal to the occipital foramen. The occipital foramen is bounded by the posterior borders of the epicranial plates which are thickened to form a collar-like band. This band is interrupted mid-dorsally and mid-ventrally where the two epicranial plates adjoin each other. On the ventral surface these plates meet in the median ventral suture, which extends from the occipital foramen forward nearly to the mentum. In line with the external corners of the mentum a strongly chitinised ridge extends backwards on each side parallel to the ventral suture (see p. 116, Fig. I E). The anterior ventral margin of the head has two triangular strongly chitinised areas produced dorsally into prolongations with which the mandibles and the maxillae articulate. There is a sensory pit on the posterior border of each of these areas (Fig. I E, *s.p.*).

The anterior clypeal border bears a pair of curved pre-clypeal hairs (Plate X, Fig. 9, *p.c.*). The pre-antennal (*p.a.*), and the inner (*p.i.*), mid (*p.m.*), and outer (*p.o.*) post-antennal hairs lie nearly in line half-way between the level of the antennal bases and the eyes. The pre-antennal (*p.a.*) and outer post-antennal (*p.o.*) are short and fan-like with about five plumose branches. The inner (*p.i.*) and mid (*p.m.*) post-antennal are long, fan-like, and have about seven plumose branches. A pair of simple sutural hairs (*s.*) lies dorsal to the eyes just within the epicranial suture. In line with these hairs, external to the suture, there is a pair of simple trans-sutural hairs (*t.s.*); and ventral to the latter,

at the dorsal margin of the eyes, arises a pair of simple ocular hairs (*o.*). On the lateral margin, in line with the outer post-antennal hairs, arises a pair of small, branched, sub-antennal hairs (*s.a.*).

The ventral surface bears four pairs of hairs (Fig. I E). Near the anterior border is a pair of post-maxillary hairs (*p.m.*). The other three pairs of hairs arise in a row midway between the anterior border and the eye level. The outer hairs (*s.b.*) are long and three-branched; a short distance internal to these is a pair of very small three-branched hairs (*s.o.*); and internal to the latter, one on each side of the ventral suture, is a pair of short simple hairs (*s.m.*). Possibly these hairs correspond to the sub-basal, infra-orbital, and sub-mental hairs described by Puri (1931) in Anopheline larvae.

Antenna. The antenna (Figs. 12 and 13) is smooth, light in colour, narrow distally and slightly bulbous for the basal third. An antennal hair (*a.*), which has about seven finely plumose branches, arises one-third of the length from the base. Distally the antenna bears five terminal sensory processes. Two of these, the inner (*i.s.*) and outer (*o.s.*) setae, are long and slender. Dorsally there is a short, stout, cigar-shaped process (*d.p.*), the distal end of which is oblique and appears to have a small central papilla. Ventrally there is a long, tapering process, the ventral cylinder (*v.c.*), which is slightly chitinised for the basal half, and very delicate and transparent distally. From the dorsal surface of the cylindrical process, near the middle, a small slender digit arises and projects forward parallel, and close to, the surface of the cylindrical process. In the centre, between these four processes, a short slender central digit (*c.*) arises.

Mandible. In dorsal view the body of the mandible (Pl. X, Fig. 1) is nearly quadrate, with the basal margin rounded, and the distal internal corner produced. The antero-lateral angle bears two long, stout, sickle-shaped hairs (*sk.*), at the base of which there is a short, stout, pointed process. In front of the sickle-shaped hairs, a ridge or collar bearing long, closely set, curved hairs arises, and curves posteriorly and inwards to form the anterior arc of hairs (*a.a.*). The distal margin is produced internally into a strong terminal tooth (*t.*), proximal to which, on both dorsal and ventral sides, there are two or three small and weak sub-terminal teeth (*s.t.*). Posterior to these teeth a soft lobular process (*l.p.*) extends inwards, and bears several tufts of fine hairs. The internal margin is formed of a strongly chitinised band which anteriorly is produced to form the anterior apodeme (*ap.*) for the insertion of the retractor muscle, and then runs backwards and divides into dorsal and ventral branches. The ventral branch is produced postero-ventrally to form a strongly chitinised articular process or condyle. In line with the anterior apodeme there is a pair of long anterior hairs (*a.h.*), and posterior to these is a group of four or five long median hairs (*m.h.*). A row of long, upwardly curved, ventral hairs (*v.h.*) arises on the anterior ventral margin. Ventral to the terminal tooth a long, stout, ventral sickle-shaped hair (*v.s.*) arises; and posterior to it there is a row of about eight slender inwardly directed hairs.

Maxilla. The maxilla (Fig. 2) is nearly quadrate; and proximally there is a small, strongly chitinised, triangular stipes (*sp.*). Laterally there is a stout maxillary palp (*p.*) nearly as long as the maxilla. The palp terminates in four sensory processes (Fig. 14). These are formed of a simple, short, outer process (*o.p.*), a similar but slightly larger inner process (*i.p.*), a long, stout, tapering, ventral process (*v.p.*), and a median process (*m.p.*). The median process arises from a well-chitinised base and then expands into a very delicate membrane, the structure of which is difficult to interpret. It appears to be in the form of a very delicate pear-shaped vesicle with strengthening chitinised ribs, but it may be formed of two spoon-shaped lamellae lying close together with the concave faces opposed.

In the middle of the anterior margin of the maxilla (Fig. 2) a curved ridge arises which bears a closely set range of long hairs forming the terminal arc (*t.a.*) of the maxilla. The ridge is extended backwards to the posterior internal corner as the median band (*m.*). This band divides the maxilla into two areas, an outer and an inner, which possibly represent the galea and lacinia respectively. On the dorsal surface of the inner area there is a curved range of long hairs, the internal arc (*i.a.*); proximal to which there is a group of fine hairs; and external to it there are fine irregularly arranged hairs. Along the ventro-internal border of the maxilla a number of long, strong, curved, ventral internal hairs (*v.i.*) arises. A group of fine hairs is present in the middle of the outer area. The maxilla bears three sensory setae. One, the median sensory seta (*m.s.*), is situated midway along the median band; another, the anterior dorsal seta (*a.d.*), is placed near the anterior margin of the outer area; and the third is on the ventral surface nearly beneath the anterior dorsal seta.

Mentum. The mentum is broadly triangular and strongly chitinised. It has a large median tooth and eight to ten teeth on each side (Fig. 11). The first five lateral teeth are small and crowded, and the posterior three are large and distant. The ninth tooth may be large or small, or absent; and sometimes there may be a small additional tenth tooth on one side.

Thorax. The thorax (Fig. I A) is twice as broad as the head and a little longer than wide. On the dorso-lateral margins there are long hairs borne on tubercles on each side. In the prothoracic region there are two simple and two branched fan-like hairs; in the mesothoracic region one simple and one large fan-like hair; and in the metathoracic region one large fan-like hair. On the ventro-lateral margins there are four tubercles. A small anterior one bears a single fan-like hair, while the other three, one on each of the pro-, meso- and metathoracic regions, bear the pleural hairs as described by Puri (1931) in Anopheline larvae (Fig. I A; P., M. and M.T.). Each of the pleural tubercles bears four hairs arranged in two pairs, anterior and posterior (Figs. 25, 26 and 27). In each case the anterior ventral hair (*w.*) is long and simple; the anterior dorsal (*x.*) is branched and fan-like with finely plumose branches numbering four, ten and seven in the prothorax, mesothorax and metathorax, respectively. The posterior pair consists of a long, simple ventral hair (*y.*), and

a very small dorsal hair (*z.*), which is simple in the prothoracic and two-branched in the others. According to Puri (1931) in Anopheline larvae the pleural hairs are in direct relation to the imaginal discs of the legs and represent the vestiges of the larval legs, following the view of Keilin (1915).

Details of the chaetotaxy of the thorax are shown in Fig. I A.

Abdomen. The abdomen is half as wide as the thorax anteriorly and tapers slightly posteriorly. In the mature 4th instar larva heavily chitinised plates are present on the dorsum of segments 7 and 8 (Fig. 3, C.P., and Fig. I A, C.P.). Sometimes a small plate is also present on segment 6. Although these plates are regarded as characteristic of the genus *Orthopodomyia*, I have found that in *O. pulchripalpis* they may be absent in some cases, and, when present, may vary greatly in size. I have observed newly emerged 4th instar larvae devoid of plates on any segment, but after a time plates appeared and gradually increased in size. The development of the plates was followed in a number of larvae. For example, a 4th instar larva was carefully examined and found to be without any trace of dorsal plates. A few days later a small bilobed plate had appeared on the dorsum of segment 8 (Fig. I B, C.P.). This plate gradually extended, and six days later reached across the segment as a saddle-like plate and a small plate had appeared on segment 7 (Fig. I C, C.P.). Seven days later, although the plate on segment 8 had further increased in size, the plate on segment 7 had grown very rapidly and was much larger than that on segment 8 (Fig. I D). MacGregor (1923) also found great variability in the dorsal plates of larvae of *Orthopodomyia arboricollis*.

Segments 1 and 2 have lateral fan-like hairs with five to six plumose branches. On segments 3, 4, 5 and 6 the lateral hairs are long and simple, and segments 7 and 8 have only small, simple, lateral hairs. In addition to the large lateral hairs, on the dorsal surface of each segment there are two anterior lateral hairs and a row of four hairs in line with the large lateral hairs on each side. The development and structure of these hairs vary in the different segments. On the ventral surface the general arrangement is a row of three hairs in line with the dorsal row and one anterior median hair.

Details of the chaetotaxy are given in Fig. I A.

Segment 8. The siphon is about four times as long as its diameter at the base and tapers slightly posteriorly (Fig. 3). A pair of ventral siphonal hairs with eleven finely plumose branches arises one-third of the length from the base (Fig. 3, *si.*, and Fig. 4). Laterally segment 8 bears a comb composed of two rows of scales (Fig. 3, *cb.*). The anterior row is formed of twenty-two scales, the median ones of which have each a ciliated base and terminate in a long curved spine (Fig. 7). Dorsally and ventrally the scales decrease in size, and the most ventral are very small and consist of a rounded ciliated lobe with no terminal spine (Fig. 8). The posterior row is usually formed of nine large scales, each with a ciliated base and a long curved terminal spine (Figs. 5 and 6). Generally the central scale is the largest and is inserted slightly posterior to the others. Dorsal to the comb there is a small two-branched hair, and, posterior to this,

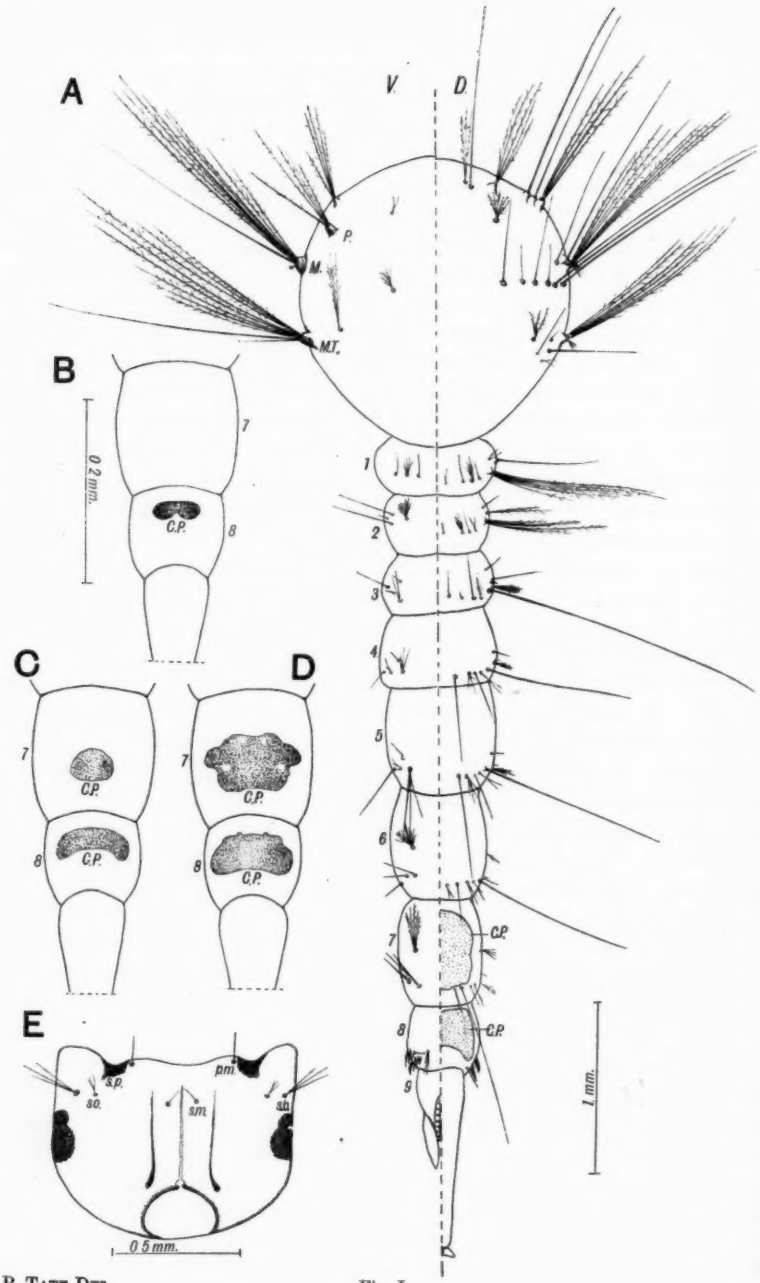

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Fig. I.

a simple hair. The sub-siphonal hair (Fig. 3, *s.s.*) is fan-like with nine finely plumose branches. A small three-branched hair arises ventral to the comb, and posterior to the ventral posterior scales there is a simple hair.

Anal segment. The anal segment (Fig. 3) is half as long as the siphon, and is encircled by a chitinised plate. On each side at its base, parallel to the comb, there is a narrow chitinised lateral band (*l.b.*). The anal gills (*g.*) are lanceolate, the dorsal pair being nearly as long as the segment and the ventral pair half as long as the dorsal. The dorsal fin (= dorsal tuft of Howard, Dyar and Knab, 1912) consists of a ventral pair of long, strong, simple hairs, and a dorsal pair of large fan-like hairs each with fourteen branches (*d.f.*). The lateral hair (*l.*) is small and simple. The ventral fin (*v.*) is usually formed of fourteen, branched, fan-like hairs arranged alternately to right and left of the median line. The anterior hairs of the ventral fin are small with three to four branches, but the posterior ones are large and have nine to ten branches.

EARLY LARVAL INSTARS.

The early larval instars differ from the 4th instar mainly in the absence of the dorsal chitinised plates on the posterior abdominal segments; the less degree of chitinisation of the anal segment; the reduction in the relative length and chitinisation of the siphon; the reduction in size and complexity of the dorsal and ventral fins of the anal segment; the structure of the comb; the number of lateral teeth on the mentum; and the simplification of the branched hairs on the head, thorax and abdomen.

3rd instar larva. The hairs on the head are arranged nearly as in the 4th instar, but the pre- and post-antennal have fewer branches (Fig. 15). Usually the inner (*p.i.*) and mid (*p.m.*) post-antennal hairs are three-branched; and the outer (*p.o.*) post-antennal and the pre-antennal (*p.a.*) are short and three- to five-branched. The antennal hair (*a.*) is situated further forward than in the 4th instar. The mentum has only seven lateral teeth (Fig. 16).

Legend to Figure I.

Fig. I A. 4th instar larva. Dorsal and ventral aspects of the right half of the thorax and abdomen, showing chaetotaxy. To the right of the dotted line is the dorsal aspect, and to the left is the ventral aspect. In each case only the hairs which are not represented in the other aspect are shown.

B. 4th instar larva, showing small chitinous plate on dorsum of segment 8.

C. The same larva 6 days later, showing increase in size of the plate on segment 8, and the appearance of a small plate on segment 7.

D. The same larva one week later than in Fig. C, showing further development of plates on segments 7 and 8.

E. Ventral aspect of head capsule of 4th instar larva.

1 to 9, segments 1 to 9 of the abdomen; *C.P.* chitinous plates on segments 7 and 8; *D.* dorsal aspect of thorax and abdomen; *M.* mesothoracic pleural group of hairs; *M.T.* metathoracic group of pleural hairs; *P.* prothoracic group of pleural hairs; *p.m.* post-maxillary hair; *s.b.* sub-basal hair; *s.m.* sub-mental hair; *so.* infra-orbital hair; *s.p.* sensory pit; *V.* ventral aspect of thorax and abdomen.

The siphon (Fig. 17) is about thrice as long as broad, the siphonal hair six-branched and plumose (Fig. 17, *si.*, Fig. 18), and the sub-siphonal hair (*s.s.*) four-branched and plumose. The comb resembles that of the 4th instar, but the scales are smaller and number only eighteen in the anterior row (*cb.*). The anal segment is less strongly chitinised, except on the dorsal surface. The dorsal fin (*d.f.*) has a pair of simple ventral hairs, and the dorsal fan-like pair has each nine to ten branches. The ventral fin (*v.*) has twelve hairs, the posterior of which have about seven branches. The anal gills (*g.*) are relatively broader than in the 4th instar.

2nd instar larva. Of the hairs on the head (Pl. X, Fig. 19) the inner (*p.i.*) and mid (*p.m.*) post-antennal are long and two-branched, the outer post-antennal (*p.o.*) and the pre-antennal (*p.a.*) are short and three-branched, and all are very finely plumose. The antennal hair (*a.*) is two-branched and is placed near the middle. The mentum has five to six lateral teeth (Fig. 20).

The siphon (Fig. 21) is equal in length to the anal segment, twice as long as broad, and is weakly chitinised. The siphonal (*si.*) and sub-siphonal (*s.s.*) hairs are three-branched. There are only fourteen scales in the anterior row of the comb (*cb.*). The anal segment is weakly chitinised except for a dorsal saddle (*d.s.*). The dorsal fin (*d.f.*) has a pair of long, simple, ventral hairs, and a pair of dorsal fan-like hairs each with six branches. The ventral fin (*v.*) is formed of six hairs each with two branches. The anal gills (*g.*) are broadly lanceolate, and the ventral pair is two-thirds as long as the dorsal.

1st instar larva. In this instar the arrangement of the hairs on the fronto-clypeus differs considerably from that of the other instars (Fig. 22). The pre-antennal hairs (*p.a.*) are long, two-branched and are situated anterior to the level of the bases of the antennae. The inner (*p.i.*) post-antennal are long and simple. The mid post-antennal (*p.m.*) are long and two-branched. The outer post-antennal (*p.o.*) are short and simple. The antennal hair (*a.*) is unbranched and is placed anterior to the middle. All these hairs are minutely plumose. The sutural hairs (*s.*) are very long. An egg-burster (*e.*) is present in a depression in the centre of the fronto-clypeus, and is formed of a strong transverse bar which is produced into a median dorsal tooth. The mentum has three small lateral teeth (Fig. 23). The mandible has a third antero-lateral sickle-shaped hair about half as long as the other two and placed behind them.

In this instar the pleural tubercles of the thorax bear two pairs of simple hairs and the fan-like dorso-lateral hairs of other instars are represented by long, strong, simple hairs.

The siphon (Fig. 24) is a little shorter than the anal segment and is weakly chitinised for the distal third. The siphonal hairs (*si.*) are two to three-branched and the sub-siphonal (*s.s.*) hairs simple. The comb (*cb.*) is formed of a single row of about eleven scales, with rounded ciliated lobes and no terminal spines. The anal segment has a dorsal chitinised saddle (*d.s.*), the surface of which is raised into minute backwardly directed scales. The dorsal fin (*d.f.*) is formed of two pairs (dorsal and ventral) of long, strong, simple hairs. There is no

ventral fin. The anal gills (*g.*) are short and broad and the ventral pair is about two-thirds as long as the dorsal.

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DESCRIPTION OF PLATE X.

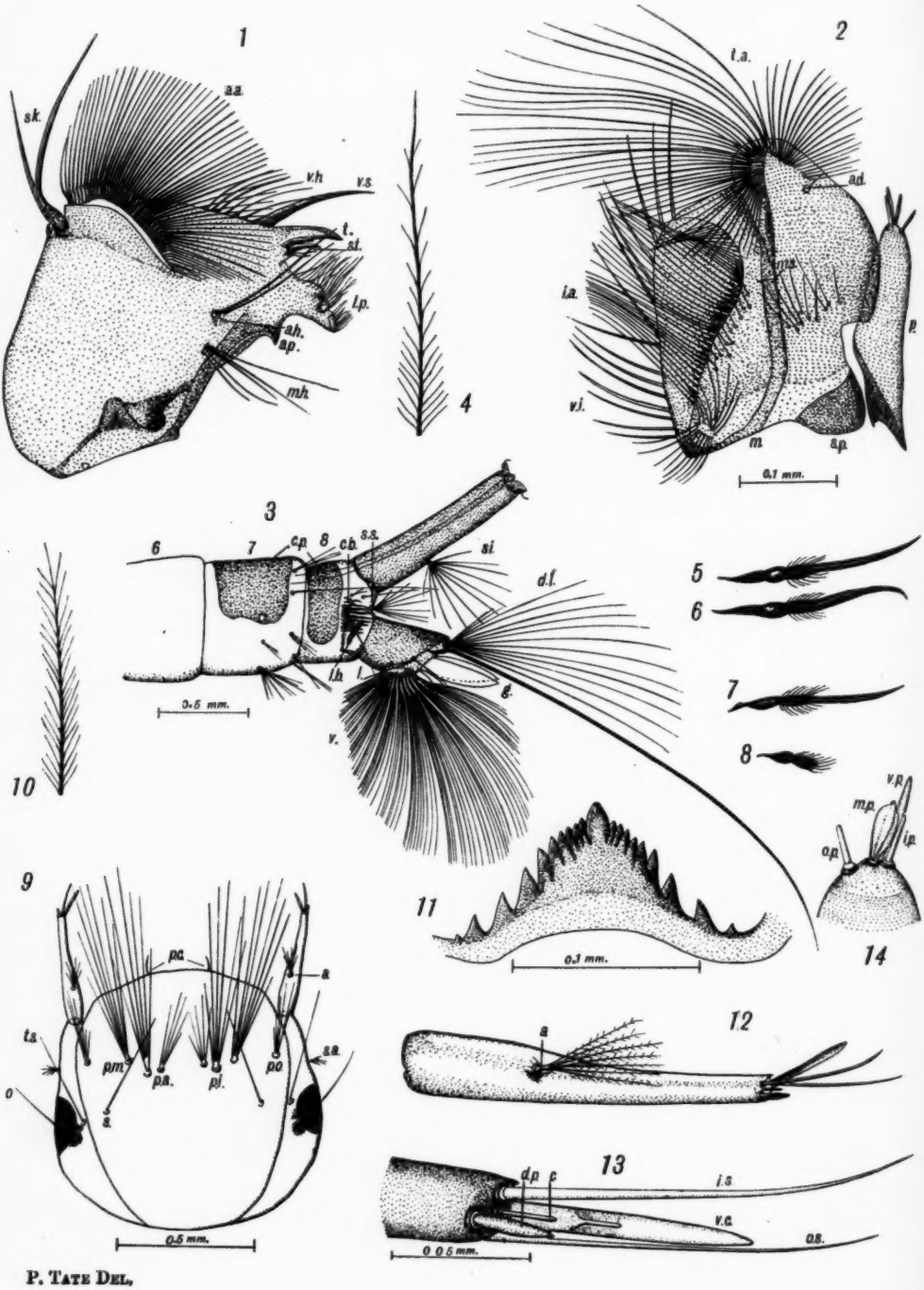
Orthopodomyia pulchripalpis. 4th instar larva.

Fig. 1. Mandible. Dorsal aspect. Scale as in Fig. 2.
Fig. 2. Maxilla. Dorsal aspect.
Fig. 3. Posterior segments of abdomen. Lateral aspect.
Fig. 4. Portion of branch of the siphonal hair more highly magnified to show pluming.
Fig. 5. Single median scale of posterior row of comb. Scale as in Fig. 2.
Fig. 6. Single ventral scale of posterior row of comb. Scale as in Fig. 2.
Fig. 7. Single median scale of anterior row of comb. Scale as in Fig. 2.
Fig. 8. Single ventral scale of anterior row of comb. Scale as in Fig. 2.
Fig. 9. Head. Dorsal aspect.
Fig. 10. Part of branch of a post-antennal hair more highly magnified to show pluming.
Fig. 11. Mentum. Dorsal aspect.
Fig. 12. Right antenna. Dorso-internal aspect. Scale as in Fig. 2.
Fig. 13. Distal end of right antenna showing sensory processes. Dorsal aspect.
Fig. 14. Distal end of maxillary left palp showing sensory processes. Dorsal aspect. Scale as in Fig. 13.
Fig. 15. 3rd instar larva. Head. Dorsal aspect.
Fig. 16. 3rd instar larva. Mentum. Dorsal aspect.
Fig. 17. 3rd instar. Posterior segments of abdomen. Lateral aspect.
Fig. 18. 3rd instar. Part of branch of siphonal hair more highly magnified to show pluming.
Fig. 19. 2nd instar. Head. Dorsal aspect.
Fig. 20. 2nd instar. Mentum. Dorsal aspect.
Fig. 21. 2nd instar. Posterior segments of abdomen. Lateral aspect. Scale as in Fig. 15.

Fig. 22. 1st instar. Head. Dorsal aspect.
Fig. 23. 1st instar. Mentum. Dorsal aspect. Scale as in Fig. 20.
Fig. 24. 1st instar. Posterior segments of abdomen. Lateral aspect. Scale as in Fig. 22.
Fig. 25. 4th instar. Base of prothoracic pleural group of hairs. Scale as in Fig. 20.
Fig. 26. 4th instar. Base of mesothoracic pleural group of hairs. Scale as in Fig. 20.
Fig. 27. 4th instar. Base of metathoracic pleural group of hairs. Scale as in Fig. 20.

Key to the lettering of Plate.

a. antennal hair; *a.a.* anterior arc of hairs of mandible; *a.d.* anterior dorsal seta of maxilla; *a.h.* anterior inner hairs of mandible; *ap.* anterior apodeme; *c.* central sensory process of antenna; *cb.* comb; *d.f.* dorsal fin of anal segment; *d.p.* dorsal sensory process of antenna; *d.s.* dorsal chitinised saddle of anal segment; *e.* egg-burster; *g.* anal gills; *i.a.* internal arc of hairs of maxilla; *i.p.* inner sensory process of maxillary palp; *i.s.* inner sensory seta of antenna; *l.* lateral hair of anal segment; *l.b.* lateral chitinised band of anal segment; *l.p.* lobular process of mandible; *m.* median band of maxilla; *m.h.* median internal hairs of mandible; *m.p.* median sensory process of maxillary palp; *m.s.* median sensory seta of maxilla; *o.* ocular hair; *op.* outer sensory process of maxillary palp; *o.s.* outer sensory process of antenna; *p.* maxillary palp; *p.a.* pre-antennal hair; *p.c.* pre-clypeal hair; *p.i.* inner post-antennal hair; *p.m.* mid post-antennal hair; *p.o.* outer post-antennal hair; *s.* sutural hair; *s.a.* sub-antennal hair; *si.* siphonal hair; *sk.* sickle-shaped hairs of mandible; *sp.* stipes; *s.s.* sub-siphonal hair; *s.t.* sub-terminal teeth of mandible; *t.* terminal tooth of mandible; *t.a.* terminal arc of hairs of maxilla; *t.s.* trans-sutural hair; *v.* ventral fin of anal segment; *v.c.* ventral cylindrical process of antenna; *v.h.* ventral hairs of mandible; *v.i.* ventral internal hairs of maxilla; *v.p.* ventral sensory process of maxillary palp; *v.s.* ventral sickle-shaped hair of mandible; *w.* anterior ventral hair of pleural groups; *x.* anterior dorsal hair of pleural groups; *y.* posterior ventral hair of pleural groups; *z.* posterior dorsal hair of pleural groups; 6, 7 and 8, segments 6, 7 and 8 of abdomen.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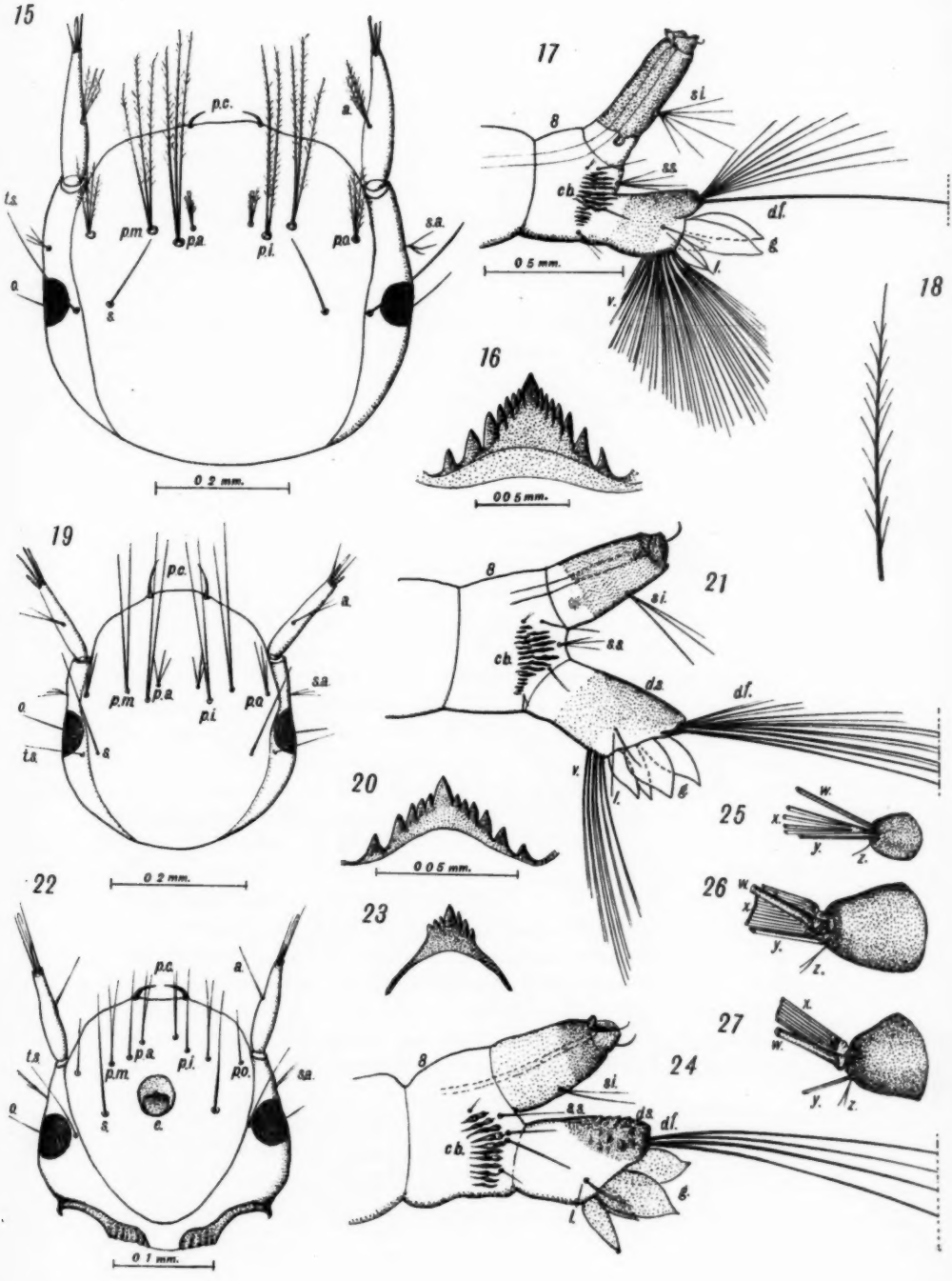
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STUDIES IN TROPISMS OF THE BED BUG *CIMEX LECTULARIUS* L.[1]

By EZEKIEL RIVNAY.

(With 9 Figures in the Text.)

Foreword.

This paper records the results of an attempt to determine (*a*) what stimuli attract the bed bug and how this insect locates its prey, (*b*) why the bug discriminates between individual persons (Kemper, 1929*a*), when it readily feeds upon animals other than man (Rivnay, 1930*b*), and (*c*) what are the factors which underlie some of the bug's habits.

Thermotaxis.

Under certain physiological conditions, some insects move toward a source of heat regardless of whether it is higher than their optimum temperature, and exhibit some definite reflex action associated with feeding. Naturally it is expected that the same organism moves away from this heat when the particular physiological conditions which brought forth the reaction have changed. To illustrate this type of reaction, the experiments of Howlett (1910 and 1917) and Marchand (1918) may be cited. These observers discovered independently that mosquitoes are not stimulated to "bite" the person by the odour of blood, nor by the odour of perspiration, but by the heat emanating from the body. Some lice react similarly. Howlett (1917) states that *Phthirus pubis* L. while feeding shows no reactions to a heated test-tube placed over its back, but "otherwise becomes greatly excited and moves with remarkable eagerness and rapidity," and "shows wild efforts to reach it." Also, Frickhinger (1916), who experimented on the body louse for olfactory reactions, came to the conclusion that the louse has a sense of heat ("*Wärmesinn*").

To my knowledge, no one has made a special study of thermotaxis with the bed bug. Occasionally, however, reference is made here and there to the effect of heat upon this insect. Hase (1917), for instance, in speaking of presumptive attracting stimuli of the bed bug, mentions first the smell of the skin and then heat. Kemper (1929*a*), also states that from a short distance the warmth radiating from the human body might act as an enticement for the insect.

Experiments.

The object of the following experiments was to determine whether or not heat is an important stimulant in calling forth feeding reactions.

[1] Being part of Ph.D. thesis, Massachusetts State College, Amherst, Mass. U.S.A.

Feeding reactions associated with heat. A test-tube 10 mm. in diameter, containing an adult female bug, was placed over the metal shade of a desk lamp so that the tube was slightly heated at one end. The bug, which had been in a quiescent state, soon reacted, directing her antennae toward the source of heat. After stroking the antennae with the fore-legs for a moment, she moved toward the source of the heat with proboscis extended, ready for piercing. Such reactions were shown also when a dissecting needle was slightly heated over a lamp and placed near a bug. Upon reaching the needle, the bug made futile attempts to pierce it. When the needle was moved, the bug followed it in every direction, but when the same needle was cooled and the procedure repeated, none of the above reactions occurred. Again, when a test-tube, 20 mm. in diameter, filled with warm water, was placed in a Petri dish containing several hungry bugs, most of them, upon detecting the heat, advanced toward it exhibiting the feeding reactions described above. All of these experiments were repeated time and again, and similar reactions were always observed. It appears obvious, therefore, that heat is an important factor in stimulating the insect to obtain food. Under its influence, reflex actions associated with blood-sucking take place regardless of whether there is a host available or not.

Influence of light, humidity and nutrition on thermotaxis. The question may be raised, what conditions influence these tropic reactions? Would light, humidity or state of nutrition affect it to any great extent? Contrary to the accepted opinion, light, when not too intense, does not seem to interfere very much with the activities of the bug. All of the experiments on thermotaxis were conducted during the day or in the evening by the light of a desk lamp—the light factor in this case being of little importance.

In order to determine whether relative humidity has any effect on these reactions, the writer tested thermotaxis under different relative humidities as follows: By means of water, concentrated sulphuric acid and various saturated salt solutions, relative humidities of different ranges were obtained. The acid, or the solution, as the case may have been, was poured into a small dish, which in turn, was placed in an air-tight jar and left there for an hour or two and in some instances for several hours. The humidity was measured by means of a dew-point apparatus which could be introduced into the jar by a special opening in its cover made for this purpose. Through the same opening, a thermometer and stirring rod could also be passed. When the desired humidity was obtained, the bugs were introduced through a very small hole in the side of the jar provided for this purpose. This opening served also for the insertion of the warm rod by means of which the thermotactic reactions were tested. This procedure was carried out under various conditions of humidity, and it was found that the insects reacted positively to heat at a range of relative humidities from about 15 to 95 per cent. However, when the object which radiated the heat was moist, the reactions were negative as shown later.

Conditions of nutrition may interfere with thermotaxis more than any of the other above-mentioned factors. Fully fed bugs do not react to heat in the

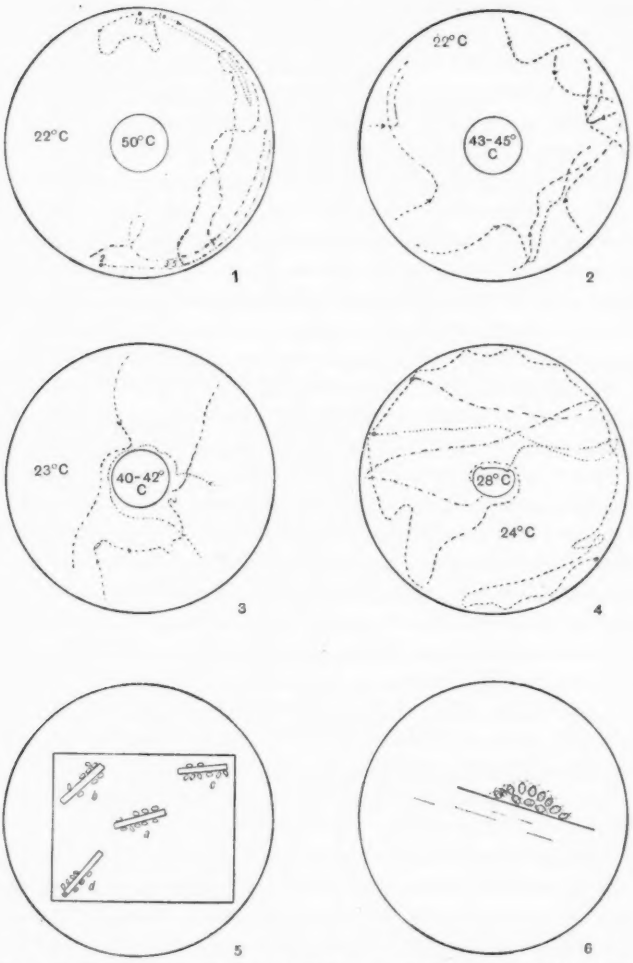
same manner as hungry bugs. Nevertheless, the writer observed, on many occasions, that some individuals, after having gorged themselves with blood, and after crawling around for a few seconds, returned to feed anew. It is not exceptional for a bed bug to manifest reactions associated with feeding on the day following its consumption of a good meal. Lack of any such reactions was noticed during the premoulting period and a day or two after moulting. The bugs which show the greatest sensitiveness to heat are those which have been starved for some time.

Influence of time and distance on thermotaxis. It is of interest to learn whether heat radiation also serves as a guide for the insect in locating its host from afar. For that purpose, it is important to establish at what distance a bug is capable of detecting heat and how quickly a response to heat takes place. The following experiments were conducted with a view to answering these questions.

A bed bug was put on a small piece of paper inside a vial 20 mm. in diameter. The vial was placed in a horizontal position and the paper, holding the insect, was placed about 4 cm. from the bottom of the vial. The latter was permitted to remain undisturbed for from 20 to 30 min. The finger was then placed against the bottom of the vial without displacing or disturbing the insect. The object was to measure the lapse of time required for the first response to heat from the moment the finger was placed against the vial. The indication of this heat detection was based upon the sudden awakening-like reaction, and the extension of the antennae. This test was repeated with several adult individuals at two different room temperatures. Summarising the measurements, we find that of ten individuals tried at a room temperature of from 22 to 23° C., on an average 1½ min. elapsed before a detection of heat was indicated. The minimum time was about 1 min., while two individuals did not display any perception of heat before the expiration of 2½ min. At a room temperature of 18–19° C., individuals under the same conditions required on an average about 3 min. or more before they detected the heat.

Other conditions remaining the same, there seemed to be no marked change in the time of reaction when the finger was placed at the opening of the vial instead of at the bottom. On the other hand, when the vial was placed vertically on the finger, other conditions remaining the same, the time of reaction was reduced to about a third. When a vial, filled with hot water of about 45–48° C., was placed in the centre of a Petri dish, along the margins of which were about a dozen bugs in an apparently quiescent stage, about the same lapse of time was required before heat detection was noticed. At 22° C., at a distance of 5 cm., from 1 to 2½ min. were needed, and at 19° C., more than 3·5 min. were required.

From the above, we learn, therefore, that bugs are not very quick in showing any response to heat, the source of which is at a distance beyond 2 or 3 cm., but when the source of heat is very close, the reaction takes place more rapidly. Upon the detection of heat, the bug does not necessarily crawl immediately to the source of heat. Often several minutes pass before it starts to move and then



Figs. 1–6. Experiments with *Cimex lectularius*. Circles represent dishes 12 cm. wide.

Fig. 1. Paths of five bugs stimulated to crawl by objects at 50° C.
Figs. 2–3. The paths of bugs about objects at temperatures indicated in the centre of the dish.
Fig. 4. Paths of three bugs approaching a human finger in the centre of the dish.
Fig. 5. Aggregation of bugs near a piece of wood.
Fig. 6. Aggregation of bugs in total darkness.

it may crawl aimlessly. The paths of a few bugs, which were very inactive and which were stimulated to crawl by heat, are shown in Fig. 1.

A vial, filled with hot water, was applied for the purpose of establishing the distance bugs crawl directly to the source of heat without wandering about. The paths of individuals about the source of heat are shown in Figs. 2 and 3.

It should be noticed that at 22° C., some bugs crawled directly to the temperature of 45° C., from a distance of 5 cm. (Fig. 2). In this case, they did not advance nearer than 25 mm. because that temperature was too high. When the source of heat was lower than 45° C., they advanced nearer to it (Fig. 3). Fig. 4 records the paths of three bugs moving about in a Petri dish, and indicate the distance at which they finally detected my finger at surface temperature, *ca.* 28° C.

At what distance can a bug detect a host? In order to create natural conditions, the writer, in his pyjamas, lay uncovered on a bed for 5 min. and then released one hungry adult bug at various distances from his body. Under these conditions the insect did not show any immediate response at distances greater than 3–4 cm.

Differential temperature required to produce reaction to heat. The following experiment was carried out to measure the differential temperature necessary to produce a thermotactic reaction. The results of the previous experiment were applied. One junction of a thermocouple of copper and constantan was placed in each of two vials, *A* and *B*, of the same size as those used in the foregoing experiment. In addition, a thermometer was placed in vial *A*. The finger was then placed at the bottom of vial *B*, about 4 cm. from the junction, while the other vial was not touched. The difference in the temperature was recorded at the end of 1, 2 and 3 min. by means of a Leeds and Northrop portable potentiometer. It was found that at room temperatures of 23 and 27° C., at the end of 1 min. the temperature in vial *B* had risen about 2° C., while at the end of 2 and 3 min. the temperature had risen 2·5°. Under similar conditions, bugs on an average reacted to heat at the end of from 1 to 2 min., therefore we may assume that a rise of about 2° C. will bring forth these reactions.

It is known that a nocturnal moth, under phototactic conditions, is liable to fly into a flame and be killed. Will a bed bug, likewise under proper conditions, because of positive thermotaxis, attempt to attack any warm object even though its temperature may prove injurious to it? From all indications, this does not seem to be the case. High temperatures may attract the bug at first, but when closer, it changes its course of direction. In the experiments described above, a temperature of 43–45° C. caused a change of direction at a distance of about 25 mm. (Fig. 2), while one of 40–42° C. caused the retreat at a shorter distance, as indicated in Fig. 3. Under the latter conditions, bed bugs were often quite active, advancing and retreating, attempting each time to touch the glass but retreating because of the high temperature.

CHEMOTAXIS.

In the literature on the subject there is a difference of opinion as to whether or not blood-sucking insects are attracted to their prey by the odour emanating from the animal. Weiss (1913), for instance, assumed that "when emerging from their winter quarters, females of *Culex pipiens* are at first positively chemotropic...." Howlett (1910), who experimented on the olfactory response

of mosquitoes, believed that neither sweat nor blood attracted them. Various medical entomologists, who have studied the olfactory reactions of the body louse, have generally obtained negative results. Frickhinger (1916), in reviewing the literature on the subject, states that most authors find that the louse does not react to odours and that they question whether the insect possesses a sense of smell. His own experiments would support this view but for one exception—wherein a body louse was attracted to a piece of cotton soaked in sweat. Kemper (1929*b*), who tested the responses of bed bugs to the odours of thirty-seven chemicals, including acids, fatty acids and other substances, states that none of the chemicals which he tested attracted bed bugs; but that the bed bug perceives and evades the odour of some fluids such as xylol, but is indifferent to others.

Assuming, like many writers, that the odour of the body is the main attracting stimulus, guiding the insect to its prey, some experiments in chemotaxis were carried out to discover which particular odour calls forth these reactions. As a positive reaction in this case, the writer designated one in which the insect manifested a reflex action associated with feeding—*e.g.* extending the antennae and proboscis in the direction of the stimulus, walking toward it, and attempting to pierce with the proboscis the object from which the odour emanated. When the insect evaded the source of odour, the reaction was considered negative. When none of these reactions was noticed, the term indifference was used.

Experiments.

After preliminary trials with xylol, which proved to be repellent, it was found that bugs detected the odour of this substance from a short distance. Only when the substance was placed within the immediate vicinity of the bugs did they retreat. Therefore, in the following experiments, in order to ensure that the insects perceived the odour of the substance tested, it was placed in close proximity to the insect.

The odour of a liquid was tested by putting a drop of it on a cover-glass and placing the same in the vicinity of bugs at various distances. Solid matter was tested by placing a piece of it about 1 cm. from the bugs. Substances such as sweat, sebum and cerumen were tested by contaminating a clean glass rod, or bone rod, with the material and placing the rod near the bugs. Hungry bugs were used in every case, unless the contrary is stated. The bugs tested were generally placed on a filter-paper in a Petri dish, and the experiments were usually carried out in medium daylight, or in the evening by the light of a desk lamp at a room temperature of 21–23° C.

Blood. When an attempt is made to study the olfactory reactions of an insect, naturally the effect of the odour of its food is tried. Although dubious as to whether the odour of the blood permeates the skin, mingling thus with the general odour of the body, I attempted, nevertheless, to test the effect of blood upon bugs.

Of 43 bugs of both sexes and different larval stages tested, in five separate groups, 23 were repelled by moist human blood, 20 remained indifferent to its presence, while 1 was repelled after first being attracted. This was apparently due to the blood being still warm when the experiment started. Similar results were obtained when rabbit and chicken blood were used. In general, although fresh, moist blood seemed repellent to the bugs, when the blood was dried, they remained indifferent to its presence. It seems, therefore, that the odour of the blood does not attract bugs and does not repel them, and that the negative reactions were perhaps due to the moistness of the substance.

Muscle and subcutaneous tissue. Muscle and subcutaneous tissue, when fresh, often proved repellent to the bugs, but on the whole they remained indifferent to them. The tissues of a rabbit, mouse and lizard were used. In only one instance did a female approach the piece of rabbit muscle with extended proboscis and bury it completely in the tissue. This piece was not warm and heat was not the attracting agent. The repulsion in the others may have been due to moistness.

Liver. A piece of bovine liver, used several hours after the slaughtering of the animal, attracted the bugs. They advanced toward it with extended probosces, but retreated at once upon coming in close contact with, or touching it. The piece of liver was not warm, and the bugs behaved in such a manner regardless of whether the liver had a moist or a dry surface. Of 14 starved bugs, 10 were attracted and then repelled. The other 4 remained indifferent.

Bile. Bile, taken from the gall bladder of the foregoing liver, decidedly repelled the bugs. Of 14 bugs of various stages tested in three groups, all were repelled.

Skin. Bugs were observed trying to feed through the dried skin of dead animals. A leg of a mouse and the ear of a rabbit attracted bugs 4 or 5 days after removal from the body. When cooled to a temperature lower than that of the room, the leg and the ear still attracted bugs attempting to feed.

These observations led to the investigation of the constituents of the skin which give rise to the odour attracting the bugs. It is understood that the skin odour in general is a mixture of odours from the corneous layer, the perspiration, and sebaceous secretion. In addition, the products of decomposition of these secretions influence the general odour to a great extent. To determine which of these odours was the attracting agent, a series of elimination experiments were carried out.

Pieces of mouse and rabbit skin were treated in two ways: (*a*) by two washings with ether, and (*b*) by washing with hot, soapy water and thoroughly rinsing in clean, hot water. After drying, the skin was used for a test on bugs. Of 15 bugs of different stages tested on the soap-washed, as well as the ether-washed rabbit skin, all remained indifferent to the skin. On the other hand, when tested on untreated skin, the same bugs reacted positively—attempting to feed. Pieces of human corneous membrane, removed from the palms of the hands of the writer, were treated as described in the previous experiment.

Of 30 bugs of different stages tested on this treated corneous tissue not one reacted positively, whereas, when tested on untreated tissue, 13 of the same bugs responded by attempting to feed, 15 were indifferent and 2 seemed to be repelled.

Perspiration. Perspiration from the body was scraped off on a cover-glass and tested on bugs. None was attracted to it. Some retreated upon approaching it closely, others were indifferent. Cotton, employed in wiping off sweat from the body, was also tried on bugs. This seemed to repel the bugs, although in a few cases some advanced toward it and attempted to feed upon the cotton. Usually, however, they were indifferent. When a glass rod was contaminated with the odour of perspiration taken from the palm of the hand and tried on bugs, the results were as follows: of 140 bugs of various stages tested in 13 different groups, 41, or 29 per cent., reacted positively, attempting to pierce the rod; 47, or 33·6 per cent., were repelled; while 52, or 37·1 per cent., remained indifferent. When the rod was contaminated with the odour of perspiration taken from the arm pit, 20 out of 28 bugs were repelled and 8 remained indifferent. The repellent agents in perspiration are probably some of the products of its decomposition. Butyric acid, which is one of these products, was tried on the insects and proved to be very repelling. This probably accounts for the fact that the sweat from the body, which goes through a longer period of decomposition than that of the hands, is more repellent to the insects. The effects of these odours upon the insects also varied with the persons who supplied the substance.

Sebum and cerumen. Another important contribution to the general body odour is that of the secretion of the sebaceous glands. These glands, with few exceptions, are located around the hair roots, and the characteristic hair odour of man, as well as other mammals, is due to the oil secreted by these glands. To test the effect of this secretion, the odour of hair was tried and proved to be attractive. Some of the bugs experimented on perceived the odour of a lock of hair at a distance of 3 cm. When the hair was washed with hot, soapy water, and rinsed with clean, hot water, it lost its attracting power.

A glass rod, contaminated with the odour of hair, was tried on 70 bugs of various stages, in seven groups: 46, or 65·7 per cent., advanced toward the rod, attempting to feed on it; 5, or 7·1 per cent., were repelled; while 19, or 27·1 per cent., remained indifferent. Similar results were obtained when the same bugs were tested on the odour of cerumen—the secretion of the external auditory meatus—which contains much sebum: 39 reacted positively, 11 were repelled, and 20 remained indifferent. The positive reactions were mostly noticed when the substances were fresh. An ether extract of human, canine and rabbit hair, prepared by the writer, did not attract any bugs, while commercial lanolin occasionally attracted a few. The sebaceous secretion, according to Howell (1930), contains a large percentage of cholesterine. This material, in its pure form, was obtained in order to test the effects of its odour upon the bed bug. While the insects usually remained indifferent to this odour, at times, especially

when used as a paste with water, a distinct positive reaction was noticed. Similar results were obtained with sodium glycocholate and sodium taurocholate.

The influence of distance and time on olfactory reactions. As mentioned above, the sense of smell in the bed bug is very weak and it cannot be compared with that of some insects which can detect food or their mates from a great distance by means of their olfactory powers. The object of the two experiments below was to measure the intensity of the sense of smell in the bug in terms of minutes and centimetres.

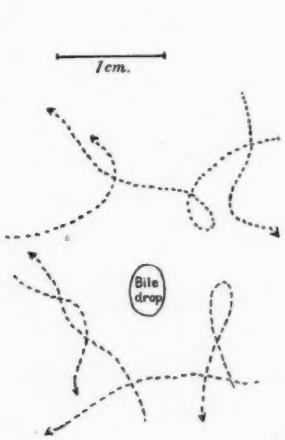
A drop of bile, which is repellent to bugs, was put in a Petri dish in which there were several hungry adult bugs. By the aid of a strong light they were made to crawl freely in the dish. The object was to see how near a bug must approach any substance before it detects its odour. The paths of the bugs verging about the drop of bile were traced while they were crawling and are reproduced in Fig. 7. As shown, the bugs were unable to detect the odour from a distance greater than 1 cm.

A few hungry adult bugs were permitted to remain on a piece of paper in a Petri dish undisturbed for about half an hour, and a lock of hair was then placed 3·5 cm. from the bugs. The object was to see how much time would elapse before the bugs perceived the odour and would begin to advance toward the hair. Six minutes passed before the first perception of the odour was indicated. The reaction was manifested by slightly raising the head, extending the antennae and stroking the proboscis with the fore tarsi. Four minutes later some of the bugs turned toward the hair and began to crawl toward it. A few advanced a short distance and then retreated; others ventured nearer to the hair. The paths of these individuals were traced immediately and are reproduced in Fig. 8. The oblong areas along the path of an individual indicate a pause of a few seconds. As noted, the paths were not straight—even at a distance of 1 or 2 cm. some individuals changed the course of direction as if searching for the source of the odour.

Experiment on the sense of taste in the bed bug. Kemper (1929*b*) states that he could not find any taste organs in the mouth cavity of the bed bug similar to those found in the mouth cavities of other Hemiptera. He adds, also, that he does not see any need for such organs in this bug since it obtains blood from a host which it has already located. McIndoo (1916) advanced the theory that insects cannot taste, while Minnich (1924) suggests that the so-called olfactory organs on the feet of some insects are organs of taste.

The following experiments in artificial feeding of the bed bug may perhaps offer some suggestions regarding the problem. By the artificial method described in a previous paper (Rivnay, 1930*a*) the writer offered the bed bugs liquids other than blood. These were: pure water, sugar solution, chicken broth and blood serum from which the haemoglobin had been removed. Of those liquids, blood serum was always the most acceptable and the bugs of all stages gorged themselves with this fluid. After they had succeeded in puncturing the membrane, all of them fed without hesitation.

Chicken broth was taken by many specimens, but in most cases only small quantities were ingested. Some individuals withdrew their probosces immediately after they had started feeding.



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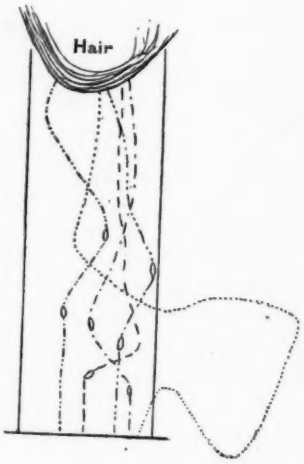

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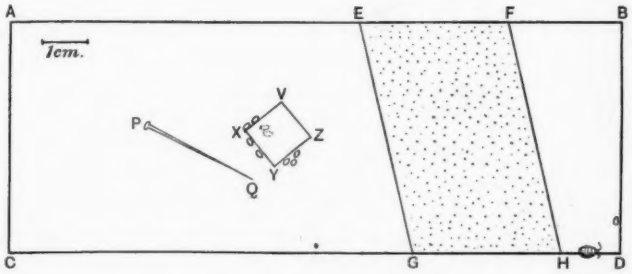

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Figs. 7–9. Experiments with *Cimex lectularius*.

Fig. 7. Paths of bugs approaching and repelled by a drop of bile.
Fig. 8. Paths of bugs attracted to a lock of hair. (Ovals along the paths indicate pauses.)
Fig. 9. Positions of bugs around a piece of botting paper opposite a lamp. [For further explanation see text.]

The sugar solution, which had been stained with Guinea Green B as an indicator, was ingested by some individuals in a quantity scarcely sufficient even to still their hunger; few obtained sufficient quantities to render the alimentary

canal green. More often the bugs refused to continue feeding after they had tasted the fluid. This tasting was indicated by a green tinge in the alimentary tract which was easily seen in larvae of the earlier instars.

Pure water was never ingested, although the writer cannot say definitely whether it was tasted or not.

HYDROTAXIS.

Very little investigation has been done in hydrotaxis with blood-sucking insects and most of our knowledge regarding these reactions rests upon casual observations rather than upon experiments. *Cimex lectularius* L., like other blood-sucking insects, shuns water. Negative reactions toward moisture were observed by the writer when adult bugs were placed therein. At first they began to walk and wade in the water, attempting to find a dry place. When the surface of the water touched the ventral side of the abdomen, they straightened out their legs and appeared as if walking on stilts. Later, when they could not keep their bodies dry, they stood still, their legs straightened out, abdomen obliquely raised and their posterior ends bent upward, managing to keep their spiracles free. In order to determine how greatly the negative reactions to moisture influence positive reactions toward heat, the following experiments were carried out.

A piece of moist paper, about 3 mm. square, was placed on the inside of a test-tube against the wall. This spot was then slightly heated from the outside by placing a finger on it. Soon, some of the bugs which were in the tube, attracted by the heat, advanced toward this spot. However, upon coming to a point about 1 cm. from the moist paper, they turned and retreated. Upon other occasions, when the paper was not present, the bugs advanced further and stroked the glass with the proboscis, as described in previous experiments. Again, when a test-tube, containing warm water at 40° C., was wrapped in a warm, moist piece of cheese cloth, they retreated before touching it.

STEREOTAXIS *v.* PHOTOTAXIS.

Bohn (1906*a*) is the only one who has carried out experiments and studied the reactions of the bed bug to light. The results he obtained, however, are doubtful. To quote an example, this author states that *Cimex* always preferred to walk in a shaded area, and that upon reaching a lighted area it turned 180°. A change in the intensity of light, according to him, brought forth a reflex action manifested by this rotation. This change in direction always occurred even though it brought the creature back to a lighted area. He believed, therefore, that "the animal is a victim of some acquired habit." In a second paper, Bohn (1906*b*) again states that every individual seems to have an inherent tendency to rotate in a definite direction, clockwise or counter-clockwise, and no matter from whence the light comes the same individual will always rotate in the same direction. None of the bugs experimented with by the present writer showed such inherent tendencies.

Experiments and Observations.

From observations in infested cages of rats and other rodents, it seemed evident that cracks, crevices and the corners between the wire netting were very much preferred to any other part of the cage. Such places, however, were quite often exposed to light and yet the bugs remained there undisturbed. In a very dark corner of a cellar of an apartment house, several specimens of bugs were found crowded in between folds and corners of an old mattress. In this instance the place was in total darkness and light could not disturb them, yet they crowded together in the folds. Generally speaking, although the bugs preferred darkness, light, when not too intense, obviously disturbed them very little. The creatures preferred to retreat to a place where they could be in contact with some object in spite of the fact that such places were often exposed to light.

Hase (1917) maintains that because of their love for the darkness, bugs aggregate, one selecting the shade of the other as a place of retreat. To quote him verbatim, he says: "Wir können sagen aus ihre Vorliebe für die Dunkelheit kommt es zu dieser Heerdenbildung der Wanzen." If this be true, we might infer that when a group of bugs are placed in total darkness, no groups will be formed. The following experiment indicates that such is not the case.

Twelve bugs of the first instar were put in a Petri dish in the centre of which a piece of glass was located. The dish was placed in a box and covered so that no light penetrated through it. About an hour later, the cover of the box was removed, when the bugs were found crowded together near the glass in the formation shown in Fig. 6. The dish was then placed in the sunlight, whereupon the larvae became restless and dispersed within a minute. This procedure was repeated, and when examined a few hours later, the bugs were again found in about the same formation as indicated in Fig. 6.

It seems to be a tendency to come in contact with some object, rather than the preference for darkness, which is the underlying cause for the formation of groups. That this positive stereotaxis is also an important factor in selecting places of retreat is shown by the following experiment.

A glass plate was placed upon some objects over a water surface. Upon this plate, a smooth piece of paper was tightly pasted, avoiding all concavities or irregularities in the surface. Although the experiment was conducted during the day, major light factors were removed by throwing a uniform shade upon the dish. Ten larvae of the second instar, which had moulted 2 days previously, were allowed to crawl freely upon the plate. After crawling for some time, and showing no signs of settling down in any particular spot, a piece of wood was placed in the centre of the plate (position *a*, Fig. 5). Within a short time, the bugs began to congregate along the sides of this piece of wood. The same bugs were brushed off and the procedure repeated three times, placing the wood in positions *b*, *c* and *d*, respectively. The bugs always grouped around the piece of wood (Fig. 5).

Should one adhere to Hase's theory, one would say that in this case the bugs came to the shade thrown by the wood. However, this is not a true explanation as shown by the following experiment.

A quadrangular sheet of paper *ABCD* (Fig. 9), 5 × 15 cm., was pasted tightly upon the flat surface of a glass plate. In order to keep the bug from entering this field, a boundary of water was drawn around it. A piece of cardboard was erected at *EF*, which threw a shade *EFGH*. The light came from a desk lamp of 40 watts, at a distance of 30 cm., at an angle of 30°. Ten larvae of the first instar were placed in this field, together with their mother. All the bugs wandered about without showing any preference for either the shaded or the lighted area. Fifteen minutes after the beginning of the experiment, a pin was placed in the position *PQ* (Fig. 9), in order to see whether the bugs would prefer to stay near it regardless of its exposure to light. Every now and then one or two of the larvae would stop for a few seconds near the pin and then resume wandering. Only one larvae stayed by it for about 8 min. and then walked away. Forty-five minutes after the beginning of the experiment, as the bugs still continued wandering, a square piece of blotting paper 1 × 1 cm. and about 1 mm. thick was placed in position *XYZV*. About 5 min. later, 2 bugs were along the side *XY*. Other larvae collected and crowded themselves against the sides of the paper trying to crawl under it. One hour after the start of the experiment, 2 larvae had crawled under the square paper; 7 were along its sides and 1 larva and the adult female were settled near corner *D*, along the margin of the paper as presented in Fig. 9.

It is clear, therefore, that not negative reaction to light, but rather positive reaction to contact, caused the selection of the place of retreat, for there was certainly more shade in the area *EFGH* than along the blotting paper. The reason the bugs did not choose to aggregate along the pin was, as noted on other occasions, because of its smooth and round surface. They usually prefer to be in contact with a rough surface.

GENERAL DISCUSSION.

Kemper (1929*b*) came to the conclusion that "the human body or any other warm-blooded animal has no enticing stimulation (Anlockungsreiz) upon a bed bug which is at a distance. This insect is capable of coming into the neighbourhood of its victim, in cases where the latter is found in a place which has been previously occupied by a host, by means of memory of the locality (Ortgedächtnis); otherwise, as it wanders at random, it may find the victim by chance. From a short distance, the heat radiating from the body may perhaps exert some influence upon it." While the present writer agrees with the first part of this conclusion, namely, that from a distance the human body exerts no influence upon the bed bug, nevertheless, he cannot accept this entire hypothesis.

As shown in the section on thermotaxis (p. 121), a differential temperature of 2° C. is sufficient to cause the bugs to crawl about. Such a differential tempera-

ture can be created by the presence of an animal even at a distance. In such cases the bugs do not necessarily crawl directly to the animal, but since it is their natural tendency to move to warmer areas, they consequently approach the host. Direct guidance by heat takes place within a very short distance from the host—(3–4 cm.). When bugs are located in a bed or mattress, little wandering may bring them into a vicinity where the heat radiation guides them directly to the host. At this close proximity the odour of the animal may have a decided influence upon the reaction of the insect, it may be repelled by, or attracted to it. Heat and odour act quite independently of each other. Insects exhibit feeding reactions under the influence of each stimulus alone; the two together cause stronger reactions.

Since the insect can be guided to the host by heat alone, one may suggest that the sense of smell in the bug serves as a means of discriminating between food and other objects. It is to be noted, however, that the insect is often repelled by an animal odour, although it may make extreme efforts to pierce objects of glass or metal which have no odour, as long as they radiate heat. As suggested by Minnich (1924) in the case of butterflies, it might be thought that the olfactory stimulation in the insect is of the nature of taste. According to him, contact or proximity of a sugar solution with the tarsi brought about a reflex action in the proboscis. In the bed bug, perception of a positive odour also causes an extension of the proboscis with an attempt to pierce the object from whence the odour emanates. However, it seems that the insect possesses the ability to discriminate between various foods only after it has taken them into the mouth cavity. It should be remembered, also, that the food of the insect exerts no olfactory influence upon it, and that the odours causing reflex actions in the bug come from an entirely different source. Furthermore, the insect was often fed artificially on occasions when the odour was of least importance, as long as it was not repellent. The chicken skin, which was employed in the artificial feeding, called forth no reaction until warm liquid was poured into the tube.

In the proximity of a host, positive reactions may be counterbalanced by other external stimuli. Humidity checks the insect from further advance; if the host is wet, the bug will not attack it. Odours unpleasant to the bug, such as stale perspiration, will equally check the insect from approaching a host. These two factors may explain why some people are attacked by bugs, while others in the same bed are not annoyed.

Upon reaching the warm object, the creature shows positive stereotactic reactions manifested at the tip of the proboscis. From observations and feeding experiments, the writer found that a bed bug makes no attempt to pierce with its proboscis a surface which is too smooth. Usually, when an object radiates heat or an attractive odour emanates from it, the bug will first stroke it with the tip of the proboscis. Should the surface of the object be slightly roughened like that of an animal tissue, it will attempt to bury the proboscis in it. On the other hand, if the surface is too smooth, like that of a finger nail, chitin, horn,

reptile scale, iron, glass, rubber, egg shell, etc., the positive tropisms gradually diminish and eventually the bug retreats. Naturally, as is the case with other reactions which diminish under a continuous stimulation, a recovery of the original reaction may take place, upon a renewed and stronger stimulation.

SUMMARY.

1. Heat is an important factor in stimulating bed bugs to obtain food. Light and atmospheric humidity do not influence the reactions to heat to any great extent, but the state of nutrition and moulting do interfere with them.

2. The bug detects heat very slowly. At a room temperature of 23° C., from a distance of 4 cm., about 1½ min. were required before a bug reacted to the heat of the hand. At a lower temperature a longer period was required.

3. At ordinary room temperature a differential temperature of about 2° C. was necessary to cause a reaction to heat.

4. Bed bugs, although attracted to heat, will be repelled when the temperature is too high. Bugs were repelled by objects at 43° C. at a distance of about 3 cm.

5. Bed bugs have a sense of smell, but only react when very close to the odoriferous substance.

6. The odours of blood, muscle, subcutaneous tissue and clean, washed skin seemed to have no influence upon the bug; the odour of bile repels them; while liver at first attracted and then repelled them.

7. The odour of perspiration, depending upon its state of decomposition, sometimes repels and at others attracts bed bugs. Of the substances tested the odours of sebum and cerumen had the greatest attracting power.

8. The bed bug apparently discriminates between foods by taste.

9. Negative reactions to water counterbalance positive reactions to heat and odour.

10. The tendency to be in contact with an object, rather than the negative reaction to light, underlies the gregarious habit of the bed bugs as well as their search for places of retreat.

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PENNELLA ZEYLANICA N.SP. A PARASITIC COPEPOD OF *HISTIOPHORUS GLADIUS* DAY

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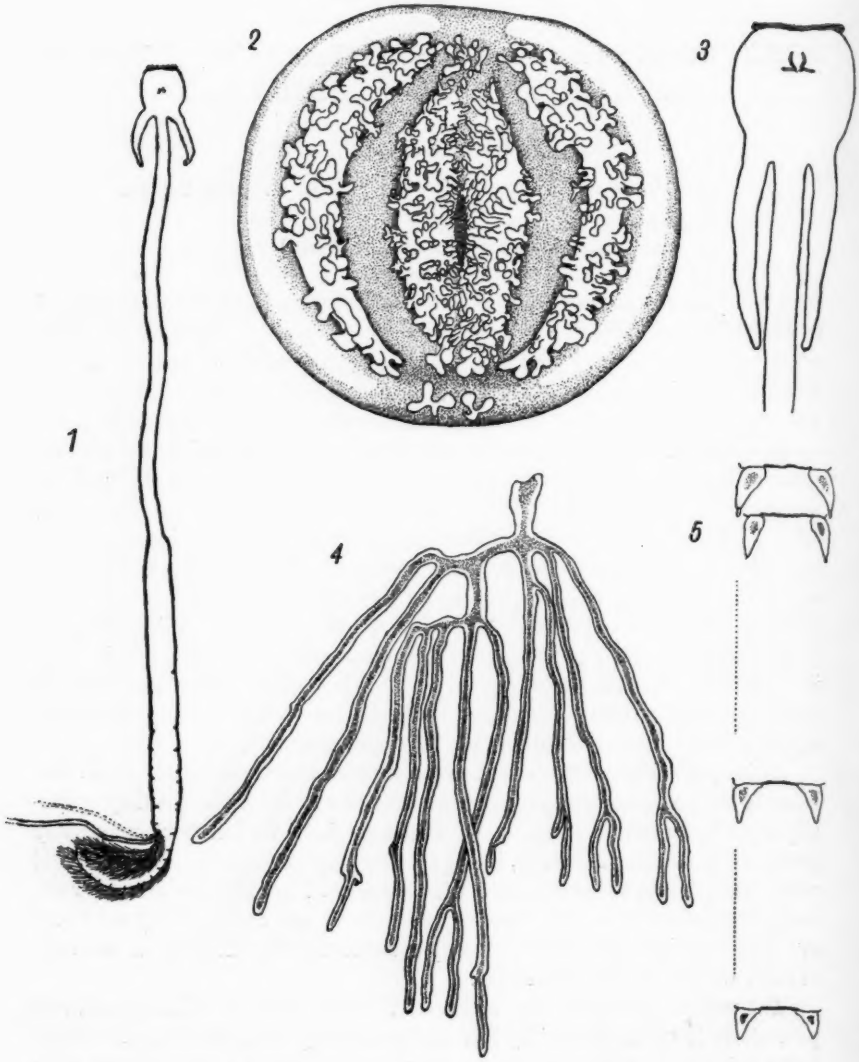
(With 5 Figures in the Text.)

Host and Record. At least four specimens of the parasite were found attached to the sides and belly of a sail-fish *Histiophorus gladius* Day caught off the south coast of Ceylon. I was fortunate enough to obtain two of these, both females, complete except for the egg strings. They measured 135 mm. and 157 mm. in their total lengths. One of them, the smaller, is illustrated in Fig. 1.

Description. Head very nearly spherical with the anterior end slightly concave and a little inclined to the ventral side. The papillae on the head are arranged in a definite pattern (Fig. 2), arising principally from two sets of soft, raised, ridges which are curved like parantheses marks, the inner set just bordering the mouth and enclosed by the outer set. The two sets are separated by an intervening space. The papillae of the outer arches are larger and not quite so densely packed as those of the inner, and in both sets of ridges there is a general tendency for the papillae to decrease gradually in size but increase in number towards the dorsal region. In addition to these there are two other small groups of papillae, one situated dorsally between the ends of the outer arches and the other ventrally in the corresponding position.

At its posterior end the head bears a horn on either side of the neck. The bases of the horns have nearly the same diameter as the neck, but they gradually taper to a rounded point. In the larger specimen the horns are practically parallel with the neck (Fig. 3), in the smaller they are slightly curved (Fig. 1) while still lying in the same plane with the neck. The chitinous skin over the horns is less thick than over the rest of the body and consequently the horns are not absolutely rigid. This probably accounts for the difference in the form of the horns of the two specimens.

The neck is long and more or less of the same diameter throughout, and posteriorly it passes insensibly into the genital segment, the diameter of the latter being twice that of the neck. Dorsally the genital segment is not separated off from the abdomen, but ventrally and also at the sides the posterior end of the genital segment stands out of the abdomen which has a diameter of about two-thirds of that of the genital segment. Transverse ridges appear at the posterior end of the genital segment. The egg strings are lacking except for the base of one on the smaller specimen. The abdomen, like the genital segment, is



Figs. 1–5. *Pennella zeylanica* n.sp. 1. Entire specimen; natural size. 2. Papillae of the head. 3. Head and horns of larger specimen. 4. Abdominal appendages. 5. Thoracic legs.

transversely ridged. Its ventral and ventro-lateral sides are covered with appendages. The mode of branching of these abdominal appendages is shown in Fig. 4, where they are spread out rather more than is natural.

The two pairs of antennae are placed close together on the dorsal side of the head. The four pairs of thoracic legs on the ventral side of the base of the neck are represented each by a basal joint, with, in the first two pairs, an additional minute segment representing the outer ramus. There is a large pigment spot on each basal joint. The first two pairs of legs are close together, the third and fourth further apart: the distances separating the legs are in the ratio 1 : 6 : 5.

Dimensions (in mm.).

	Specimen A	Specimen B
Head	7	7
Neck	59	63
Genital segment	44	55
Abdomen	25	32
Entire length	135	157

Remarks. It is not possible to identify these specimens according to Wilson's key to the species of the genus *Pennella* or to the more recent revision of the genus by Leigh-Sharpe (1928). In the arrangement of the papillae and the nature of the horns these specimens resemble *P. instructa* Wilson, a parasite of the swordfish, but they differ from it in the characters tabulated below:

	P. instructa	*P. zeylanica*
Head	Longer than wide, squarely truncated anteriorly: lateral walls slightly concave due to swellings at the bases and at the anterior truncated end	Very nearly spherical; surface smoothly convex
Antennae	First pair separated from second by a considerable distance	Both pairs close together
Thoracic legs	Only basal joints present; distances between them = 1 : 5 : 6	Basal joints of the first pairs retain vestiges of the outer rami; distances between legs = 1 : 6 : 5
Abdominal appendages	Dichotomously branched	No clear dichotomy; branching irregular

These differences together with the difference of hosts (which is in itself not conclusive) are in my opinion sufficient to warrant the separation of these specimens into a new species, *P. zeylanica*, closely allied to, though distinct from, *P. instructa*.

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A NOTE UPON *TRICHOMONAS SANGUISUGAE* ALEXEIEFF 1911

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(With 1 Figure in the Text.)

Alexeieff (1911) described two trichomonads from the horse-leech *Haemopis sanguisuga: Tetratrichomonas prowazeki* and *Trichomonas sanguisugae. T. prowazeki* is characterised by its four anterior flagella of unequal length and its poorly developed axostyle. The feature which he considered to be of specific value in *T. sanguisugae* is the "chapelet de grains" in place of the basal fibre.

T. sanguisugae was cultivated by Drbohlav (1925) in agar covered with distilled water or ovomucoid liquid, but he gave no description of its detailed structure or of its method of division. I found *T. sanguisugae* in the hind gut of *Haemopis sanguisuga*[1] and cultivated it in a medium consisting of either inactivated horse serum 1 : 10 in 0·5 per cent. NaCl solution + a little solid sterile rice starch, or an inspissated horse-serum slope covered with the liquid medium. I found that, like *T. batrachorum* and *T. hominis* (Bishop, 1931), *T. sanguisugae* will ingest rice starch grains, though its normal diet is bacteria. During the four months I cultivated this species, though I have frequently examined cultures of all ages, I have never found cysts.

In all the preparations which I have examined the anterior flagella have been three. They are approximately one and a half times the length of the body and arise from the blepharoplast which consists of a compact mass of three or four granules. The body varies in size (average 10 × 7 microns). The axostyle projects approximately 5 microns beyond the end of the body. The average length of the body therefore from the anterior tip to the posterior end of the axostyle is 15 microns.

The axostyle (Fig. 1 A) is well developed. It arises from the blepharoplast, runs alongside the nucleus and projects, tapering gradually to a point, from the posterior end of the body. In the resting stage the oval nucleus contains a well-developed central karyosome surrounded by a tiny halo. There is a well-defined nuclear membrane and few or no extra-karyosomic chromatin granules

[1] For the identification of the leeches I have used the paper by Harding (1910) and have adopted his nomenclature.

are present in the resting nucleus. In place of the basal fibre as found in *T. batrachorum* and other trichomonads (Bishop, 1931), *T. sanguisugae* contains a row of granules (sometimes they are slightly irregular and almost form a double row) which, like the basal fibre of other species stain intensely with iron haematoxylin. The posteriorly directed flagellum is attached to the body edge by a delicate membrane and projects freely for about 10 microns beyond the end of the body. The loops of this flagellum and the undulations of its membrane are very pronounced.

The mouth is very large and well defined in fixed preparations. It appears to have a delicate fibril, or siderophilic edge, on the lip distal from the nucleus.

The cytoplasm is frequently filled with food inclusions.

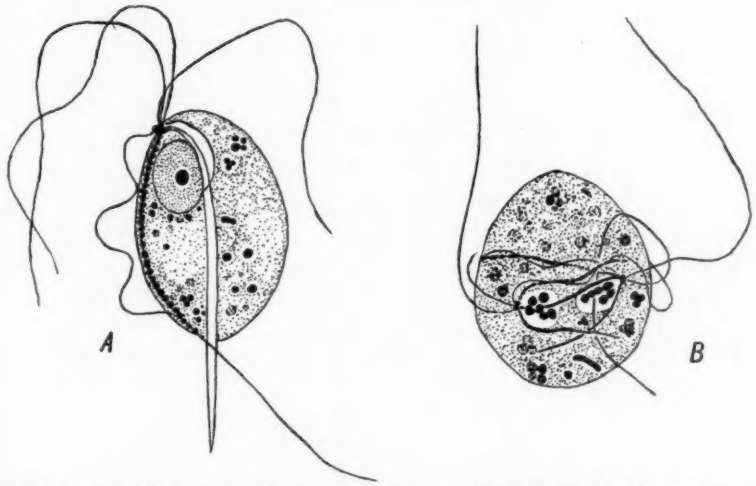

Fig. 1. *A. Trichomonas sanguisugae. B*=telophase. ×3300. For explanation see text.

I have found all stages of division. They are similar to those of *T. batrachorum* (see Bishop, 1931). Six large chromosomes of unequal size are formed in the prophase. The new string of granules (homologous with the basal fibre) is formed as an outgrowth from the blepharoplast and later a new flagellum and its membrane are formed along it. The blepharoplast, which has become a compact rounded granule, divides, and the daughters move apart forming a centrodesmose between them. The axostyle disappears and the body rounds itself off before the metaphase. At the metaphase the blepharoplast at either pole appears to divide to form a centrosome and true basal granule, but this is not nearly so clear as in *T. batrachorum*. At the anaphase there is a single blepharoplast again at either pole. The chromosomes divide and the daughter granules move towards the poles. New axostyles are formed (Fig. 1 B) by outgrowths from the daughter blepharoplasts. The centrodesmose joining the daughter blepharoplasts persists until the body divides, when it breaks

through and is subsequently absorbed. New flagella grow out from the daughter blepharoplasts as in *T. batrachorum*.

The presence of the "chapelet de grains" is persistent throughout division and in all individuals and is of specific importance. In all the details of division *T. sanguisugae* agrees with *T. batrachorum*.

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